Didymosphenia geminata; an example of a biosecurity leak in New Zealand

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"Although no one is likely to get into New Zealand again
accompanied by a live red deer, we have to accept
the proposition that invasion of animals and plants and
their parasites and our parasites will continue as far as the next
Millennium and probably for thousands of
years beyond it."
(Elton 1958)

Didymosphenia geminata on a natural substrate under water

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Abstract

Didymosphenia geminata is a diatom that has been accidentally introduced to New Zealand's South Island rivers. It has grown to bloom conditions in all rivers it inhabits, which has caused impacts to the river systems, loss in recreation value, and economic losses. The pathways and vectors of dispersal are difficult to control and hence it continues to spread throughout the South Island. Laboratory experiments assessed the survivability of *D. geminata* in different environmental conditions, a range of combinations of light availability, temperature and moisture. Experiments in the field were based in the Waitaki River to determine growth rates of *D. geminata*.

D. geminata is growing in a greater range of temperature and light conditions than previously recognised. In cool to cold conditions with a little water this diatom can survive up to 1500h, the colder temperatures also increase survivability in the dark. However, *D. geminata* has reduced survivability in warm, damp conditions, up to 60h. In the Waitaki River *D. geminata* is attaining biomass of 2.51mg mm^{-2} over six weeks during summer. This high biomass is causing a change in biotic and abiotic conditions. Longevity of survival and the range of conditions in which it can survive increases the risk of spread throughout New Zealand and the world.

There are considerable problems with invasive species and international trade. Policies aiming to reduce international invasions due to trade are becoming more prevalent as the consequences of invasion are more obvious and costly. New Zealand has been able to implement polices in the last decade that has reduced the number and variety of incursions. However these policies did not stop *D. geminata* arriving. This shows that even with the best policies species can invade fragile ecosystems. Central and local government policies surrounding management of invasive aquatic species were reviewed. Central and local government policies were developed to reduce the spread of *D. geminata*, however they are not effective as the diatom is still dispersing. Further research is required to elucidate means of dispersal in New Zealand, in particular the importance of dispersal by animals compared with that by humans, and the importance of continuing international dispersal.

Chapter 1: Introduction

1.1 *Didymosphenia geminata*, a New Zealand biosecurity failure

Now more than ever, biosecurity and invasion biology are important locally and globally. Historically, there has been worldwide movement of species through natural and anthropogenic means (Mack 2003). Recently the rate of species arrival at distant destinations has increased with international trade (Coutts and Taylor 2004). As Mack, Simberloff, Lonsdale, Evans, Clout, and Bazzaz (2000) so aptly put it "an ounce of prevention is worth a pound of cure", which is entirely applicable to biological invasions.

New Zealand has a world renowned approach to managing biosecurity, but species are still able to enter and establish. In October 2004, *Didymosphenia geminata* (Lyngbye) Schmidt, a diatom, was found in the lower Waiau River (Kilroy 2004). It has spread in the last three years to 53 rivers and lakes in the South Island covering nine catchments. Without careful management and research it will spread much further into New Zealand's most pristine river environments.

1.2 The economic costs of biological invasions

The United States of America (USA) spends over US$120 billion dollars per year due to losses and damage caused by, and control of, non-indigenous species (Pimental, Lach, Zuniga and Morrison 2000, Levine and D'Antonio 2003). As an example, the USA has an estimated 63 million pet cats and 30 million feral cats. Feral cats alone cause US$17 billon per year in damage to bird populations. Pet cats are reported to do similar damage. This cost does not include the many small mammals, amphibians, and reptiles that are killed annually by pet and feral cats (Pimental *et al.* 2005).

Neubert and Parker (2004) found that, at least initially, the cost associated with the damage caused by invasive species increased with the length of time over which they had been established. Costs were suggested to be proportional to the area occupied and growth rate of the invading species. The remediation and removal costs also increased over time (Neubert and Parker 2004).

1.3 Two examples of biological invasions

The human-mediated movement of organisms over the past 200-500 years, deliberate and accidental, undoubtedly dwarfs in scope, frequency, and impact the movement of organisms by natural forces in any previous 500 year period (Mack *et al.* 2000). The prominence of deliberately introduced species that later became invasive pests emphasizes that not all pests arrive inconspicuously, but that many are the product of deliberate but disastrously flawed human forethought (Mack *et al.* 2000).

An example of invasion[1] via ships is the movement of Eurasian zebra mussel (*Dreissena polymorpha*) to North America in ballast water. On arrival, the mussels spread rapidly, covering the bottoms of lakes, rivers and moving into industrial waterworks (Andersen Adams, Hope, and Powell 2004). It now costs billions of dollars per year to clear intake pipes of this mussel. The presence of zebra mussels has reduced natural algae populations (Barberio, Rockwell, Warren and Tuchman 2006), and increased concentrations of nutrients through entire ecosystems (Vitousek D'Antonio, Loope, Remanek and Westbrooks 1996). These environmental modifications will change the diatom assemblages to species which have superior competitive abilities under the new conditions (Barberio *et al.* 2006).

Goats (*Capra hircus*) are an example of deliberate introductions due to disastrously flawed human forethought. They were first released in New Zealand in 1773 by James Cook at Queen Charlotte Sound and Hawkes Bay (Cochrane 1994). Goats were used as a food source for sealers and whalers, and supported the mohair industry. By 1937, they were a pest species and the government sponsored feral goat control. They were causing widespread damage to native vegetation by eating a wide range of plant species. Other ecosystem impacts included debarking of trees, changes to plant communities due to loss of palatable species and, possibly, facilitation of invasion by weed species (Cochrane 1994).

1.4 Invasion theory

There are many hypotheses which purport to explain the process of invasion (Williams and Fitter 1996, Heger and Trepl 2003, Pauchard and Shea 2006). These hypotheses have been

[1] Definitions and terms are located in Appendix 1

applied to the prediction of invasions (Smith, Lonsdale, Fortune 1999, Daehler and Carino 2000, Davis, Grime, Thompson 2000, Shea and Chesson 2002, Heger and Trepl 2003, Floerl and Inglis 2005). As Williams and Fitter (1996) pertinently said, each pest is a pest for its own reasons.

The "steps and stages" model, regards invasion as a process in which a species must advance from one step and stage to the next (Heger and Trepl 2003). "The patterns of invasion for species are scale dependant. At each step in the invasion process there are scale dependant hurdles to overcome." This model is summarised as:

- Step one, immigration. Stage one, presence in the new area.
- Step two, independent growth and reproduction. Stage two spontaneous establishment.
- Step three, growth to minimum viable population. Stage three permanent establishment.
- Step four, colonisation of new localities. Stage four spreading in the new area is complete.

Pauchard and Shea (2006) agreed that each step in the model is scale dependant. Furthermore, Williams and Fitter (1996) agreed with the four stages to invasion for a species. However, they used the terms: imported, introduced, established and pest for the four steps, and the three transition stages are described as: escaping, establishing and becoming a pest.

There can be a lag phase between being an established species (stage three) and a pest (stage four). Some species like the zebra mussel have only a brief lag phase or none at all (Mack *et al.* 2000), whilst many established species do not become abundant or widespread for decades (Ricciardi and Cohen 2007). This variability in lag phases makes it hard to distinguish potential invaders from other recent introductions which will not make it past stage three. It is unclear if *D. geminata* had a lag phase as the time of establishment is unknown.

1.5 Niche theory

Invaders can utilise existing niches and exploit them to their benefit or they can engineer the environment creating new niches and conditions. Invading ecosystem engineers can alter

ecological properties (Bright 1999), such as the ecosystem's physical features, nutrient cycling, and plant productivity (Mack *et. al.* 2000, Andersen *et al.* 2004, Riccardi and Cohen 2007).

American chestnut (*Castanea dentata*) is an example of invaders utilising existing niches. First, in the mid 1800s Phytophthora root rot (*Cryphonectria parasitica*) was imported to America accidentally from Asia, which caused widespread death of the American chestnut trees (Rossman 2001). Second, in the early 1900s chestnut blight arrived causing the branches to canker (Anagnostakis 2001). It arrived in chestnut trees imported as ornamentals from Japan and China in the early 1900s. Third, in 1974 the oriental chestnut gall wasp arrived on cuttings of Chinese chestnut from Asia, which managed to avoid plant quarantine. The gall wasp causes reduction in fruiting, suppresses shoot elongation, and trees with severe infections lose vigour and often die (Anagnostakis 2001).

An example of an ecosystem engineer is the nitrogen-fixing tree *Myrica faya* in Hawaii. *M. faya* seeds are dispersed by birds to new sites created by volcanoes, which are typically nitrogen deficient. The increase in nitrogen alters the native assemblages of flora and fauna that thrive in nitrogen deficient conditions (Vitousek *et al.* 1996, Vitousek, D'Antonio, Loope, Remanek, and Westbrooks 1997). Additionally, *Melaleuca quinquenervia* in Florida is spreading at a rate of 50 acres per day through the Everglades ecosystem. It was introduced as an ornamental curiosity, and is now destroying valuable natural resources, reducing freshwater supply and creating a severe fire hazard (Rossman 2001).

1.6 Predicting invasion

There are many models and theories that attempt to predict invasion. Shea and Chesson (2002) suggested that invasion can be predicted using community ecology. Their model incorporates responses of species to natural enemies, the physical environment of the community, resource availability and the presence or absence of niche opportunities. Heger and Trepl (2003) classified this as a Key-lock Model and assumed that characteristics of invading species are suited to the specific conditions in the new environment.

A contrast to the Key-lock Model is the Fluctuating Resource Theory (Davis *et al.* 2000). This theory focuses on community susceptibility to invasion. This susceptibility is not a static or

permanent attribute, but a condition that can fluctuate over time. They suggested that functional attributes of exotic species are similar to native species. This is why when the attributes of exotic species and natives are compared the two groups are functionally indistinguishable. The theory of fluctuating resource focuses on the opportunities for invading species to capture resources which can be limited in space and time.

Floerl and Inglis (2005) showed that site susceptibility to invasion of non-native species is due to the species (via habitat associations that allow invasives to take advantage of the transport vector) and the vector itself (through direct management of specific sites and border control). Mack *et al.* (2000) also pointed out that the likelihood of a community receiving immigrants is influenced by its proximity to a point of entry. This proximity may also affect the frequency, speed, and mode of dispersal of immigrants.

Screening programmes aim to reduce the number of pests that are intentionally introduced in different regions of the world. Each programme is customised to a specific country and/or ecosystem in order to combat specific invasive species. It incorporates life history, biogeography, habitat characteristics and pest history data (Daehler and Carino 2000).

The Australian model of screening programmes has proved to be the most accurate and versatile. It has been modified slightly for use in New Zealand (Daehler and Carino 2000). Smith *et al.* (1999) stated: "The probability of a species becoming a pest is 0.1% of all introduced species", as these events are rare it is difficult for screening programmes to forecast them reliably. Smith *et al*. considered that there is a trade-off between the difficulty of controlling an established species and the margin of unreliability in screening programmes. They further suggested that any intentional establishment of species into marine and freshwater systems should not be allowed due to the irreversibility of introductions.

1.7 Pathways of invasion

Mack (2003) explained that there are natural pathways and those developed by humans. Species are moved along natural pathways such as the Gulf Stream by natural forces such as ocean and wind currents. The Gulf Stream carries seeds and plant propagules to the British

Isles. Although Mack (2003) cautioned that these natural forces are not feeble, just infrequent in their global impact.

Human-mediated pathways historically followed natural currents until the advent of steam-powered, trans-oceanic ships. As a result, species were introduced to new ranges that they would not have reached by ocean currents alone (Mack 2003). Elton (1958) demonstrated that on one day in March 1936 the British Empire fleet had 1,462 ocean-going vessels at sea and a further 852 in port. The numbers of vessels and their associated cargo at sea and in port today are considerably more. Furthermore, Elton mentioned in 1929, he found 41 animal species, mostly insects, on a Rangoon rice sailing ship from Trinidad to Manila. Many of these insects were found inside luggage when unpacking. Powel (1968) noted in New Zealand from 1963-1967 over 1,500 items of botanical interest were intercepted by port authorities. Of this total 65 were listed as harmful species, 45 of these harmful species had not been recorded in New Zealand before.

International trade "leaks" exotic species (Thompson Campbell 2001). Many insects, plants and pathogens hitch a ride through imported cargo. The dispersal of non-indigenous species mostly occurs unintentionally through packaging, on vehicles, as passengers on traded goods or as objects of trade (Levine and D'Antonio 2003, Margolis, Shogren and Fischer 2005). The resulting invasions from unintentional introductions are a market failure which is rooted in international trade (Margolis *et al.* 2005).

There are number of modes by which plants and other organisms can migrate across geographic barriers. The most common vector for plants is through the ornamental trade (Perrings, Dehnen-Schmutz, Touza, and Williamson 2005), by the contamination of potted plants with other plants, seeds and pathogens. Other pathways include the horticultural and cut flower trades in which produce is often contaminated with insects (Reichard and White 2001, Perrings *et al.* 2005, Work, McCullough, Cavey and Komsa 2005).

1.8 Propagule pressure

For each invasive species there is a minimum viable population or threshold in the new region or location (Kowarik 2003). There are two ways in which a minimum viable population can be

achieved. It can occur through a single incursion consisting of the minimum population, or through a number of incursions reaching the minimum population (Simberloff 1989, Williams and Fitter 1996).

As a general rule, with increasing propagule numbers the chance of establishment will increase (Williamson 1996, Williams and Fitter 1996, Kowarik 2003, Floerl and Inglis 2005, Von Holle and Simberloff 2005, Pauchard and Shea 2006). Repeated introductions of invasive species can occur through frequent transport to a location from the same source or via transport from multiple infested sites. Each successive establishment or secondary release increases the number of satellite populations from which the species can then spread naturally (Kowarik 2003, Floerl and Inglis 2005). This pattern of establishment and spread is one of the major drivers of successful invasions, and can be uncoupled in time and space (Kowarik 2003).

The minimum viable population number can be very small as in the case of the gypsy moth *(Lymantria dispar)* in Massachusetts. Gypsy moths were brought in once by an enthusiast in the mid 1800s. A few eggs and/or caterpillars escaped and started the continuing epidemic (Elton 1958).

Alternatively, the minimum viable population number can be large as in the case of the brushtail possum (*Trichosurus vulpecula*) in New Zealand, a number of introductions were necessary for the population to become stable. Possums were imported officially 28 times from Australia by the acclimatisation societies and private citizens. They were liberated at many destinations in New Zealand from Kerikeri in Northland to the Auckland Islands in the South. On average there were ten animals brought to New Zealand at each liberation, but this ranged up to 42 animals on particular occasions (Pracy 1962).

From 1858-1920 possums were a protected species but after 1921 possums were hunted for their fur (Mc Dowall 1994). At this time the government and acclimatisation societies were informed that possums were beneficial for forests, they provided fur and were unlikely to become pests. During the 1930s possums were hunted heavily, so much so that there was a ban on hunting to allow them to recover (Mc Dowall 1994).

There is now an estimated 70 million possums in New Zealand, and they consume seven million tonnes of plant material every year[2]. Possums are the main vector for bovine tuberculosis in cattle as they are a reservoir for the disease (Arthur *et al.* 2004). New Zealand spends over $NZ 50 million a year in pest management for this species, and millions more on developing eradication and control techniques[3].

1.9 Invaders and modified environments

There are indications that invasive species have no specific properties that provide a commonality between all pest species. However, the modification of abiotic and biotic environments can enhance the possibility of species invading more habitats (Pauchard and Shea 2006). Human alterations of disturbance regimes promote the invasion of species that would otherwise not be found in a region (Mooney and Drake 1989, Vitousek *et al.* 1996, Kowarik 2003). Some invasive species also promote an increase in disturbance, such as invasion of grasses which promote fire (Mack *et al.* 2000).

Grass species provide additional fuel for fires and they can cause a microclimate that favours increased frequency of fires. The change in disturbance regimes selects for fire-adapted species and then creates a positive feedback system that perpetuates low diversity shrublands and savannah (Vitousek *et al.* 1996, Vitousek *et al.* 1997). Mack *et al.* (2000) suggest that ecosystem transformation can be so complete that the landscape itself is profoundly altered, from open forest and savannah to grasslands.

The suppression of disturbance regimes can also promote invasions of non-native species. For example, damming of rivers changes flood patterns to consistent flow. This causes invasions into stream banks, rivers and flood plains. Invaders can reduce the biodiversity of the system by increasing the number of permanent tertiary species (Bisson, Rieman, Luce, Hessburg, Leed, Kershner, Reeves, and Gresswel 2003).

[2] www.doc.govt.nz/templates/page.aspx?id=33423 16/5/2007

[3] http://www.maf.govt.nz/mafnet/publications/research/biological-management-of-possums/biological-management-of-possums-16.htm#P2048_233216 16/05/2007

The conversion of dense forest to pasture of introduced grasses in Amazonian Brazil has ecosystem and global level impacts. The reduction of biomass and sequestration of carbon dioxide can potentially influence global climate (Mack *et al.* 2000). The lower evapotranspiration from grasslands could also translate into greater convective heat loss and increases in air temperature. Fire and land-clearing initiate these changes in the Amazon, the persistence of grasses then limits any natural re-colonisation and regeneration (Mack *et al*. 2000).

1.10 Examples of invasive macrophytes

1.10.1 *Caulerpa taxifolia*

Caulerpa taxifolia was initially planted in the Mediterranean below the Monaco Oceanographic Museum in 1988 in order to supply the aquaria with algae (Meinesz 1999, Kowarik 2003). This marine macrophyte alga is native to tropical areas, on coral reefs in the Caribbean and Polynesia (near Tahiti). *C. taxifolia* now covers thousands of hectares around Mediterranean coasts (Meinesz 1999) as eradication and control was delayed due to political arguments (Simberloff 2003).

C. taxifolia is an example of rapid asexual reproduction of an invasive species. A single propagule (small cutting) was the origin of thousands of thalli which were distributed by specialists and curators around public aquaria in Europe. This clone became cold tolerant in aquaria, this facilitated its growth in Mediterranean conditions. Individuals directly transferred from the tropics would not tolerate Mediterranean conditions (Meinesz 1999).

1.10.2 *Lagarosiphon major*

Lagrosiphon major (Ridl.) is a vascular water weed that has established in a number of lakes around New Zealand. *L. major* originates from Southern Africa where it is found in high mountain streams and ponds (Howard-Williams and Davies 1988). It was first reported in New Zealand in the Hutt Valley in 1950, by 1957 it was an established pest in Lake Rotorua. In the 1980s *L. major* had invaded Lake Taupo, Lake Rotoiti, Lake Rotorua and Lake Tarawera (Howard-Williams and Davies 1988, Wells, De Winton and Clayton 1997). Wells *et al.* (1997) suggest that dispersal had human vectors, most probably inter-lake boat transfer.

L. major has caused a reduction or replacement of native flora in many areas of Lake Taupo. Swans gather at Lake Taupo to graze the water weed, they graze to their maximum depth then move to shallow water to graze and uproot native plants. Dense populations of freshwater crayfish (*Paranephrops planifrons*) graze on characean meadows, these meadows have become scarce to due *L. major* suppressing growth and the intensive grazing by the freshwater crays (Howard-Williams and Davies 1988).

1.11 Algal invaders of New Zealand's freshwater habitats

1.11.1 Water net

In 1986, the green alga (Chlorophyta), commonly known as water net (*Hydrodictyon reticulatum* (Linn.) Lagerheim) was first reported in Tauranga in an ornamental pond (Hawes and Smith 1993, Wells, Hall, Clayton, Champion, Payne, and Hofstra 1999). The alga then spread to Lake Rotorua and Lake Rotoiti. Eight years after being first reported water net had spread to its maximum extent, throughout the Bay of Plenty and Waikato regions. Its impacts included: extensive surface floating mats (up to 30 ha), clogged hydro-station screens and filters, de-oxygenated water, entrapped fish and smothered plants (Wells *et al.* 1999). By early 1995, water net had started to decline to a point where it was no longer a pest. It is still present in some lakes and rivers but not in bloom proportions. The decline is largely unexplained (Wells *et al.* 1999). This pattern of establishment, rapid increase, maintenance, and then entire disappearance or reduction to a remnant population, is not rare (Simberloff and Gibbons 2004). The aquatic angiosperm *Elodea canadensis* showed this pattern in Great Britain from initial establishment in 1842 to decline in the early 1900s.

1.11.2 *Didymosphenia geminata*

Didymosphenia geminata has invaded New Zealand and established in pest proportions. *D. geminata* is a microscopic diatom which adheres to rocks and other river substrates by mucilaginous stalks. The original habitat preferences of *D. geminata* are high altitude areas of the United Kingdom, which are fast flowing, cold, oligotropic alpine rivers (Kawecka and Sanecki 2003). Optimal growth occurs where large rocks and boulders form the river bed (Kilroy 2004). High light conditions are necessary for large blooms, but *D. geminata* is still able to grow successfully in low light conditions (Kilroy, Lagerstedt, Davey, and Robinson 2006). After establishment of the initial cell, the stalk and attached cell then divide by

vegetative reproduction to form a small colony with branched stalks (Kilroy 2004). The small colonies then join together to form a thick mat over river bottoms when in bloom (Fig.1.1).

Fig.1.1. Waitaki River on Septermber 15th 2007, the entire shore line is covered in dense *D. geminata.*

D. geminata also occurs throughout North America (Kawecka and Sanecki 2003). In Europe it is found in low numbers and is used as an indicator species of unpolluted healthy rivers (Kawecka and Sanecki 2003). Currently, *D. geminata* has been found only in catchments of the South Island of New Zealand. It was first found in the Mararoa and Waiau Rivers in Southland. It spread quickly to the Buller and Hawea/Clutha catchments. Figure 1.2 indicates the rivers and catchments that are currently contaminated with *D. geminata*. Notably, some lakes have also become contaminated such as Lake Wakatipu.

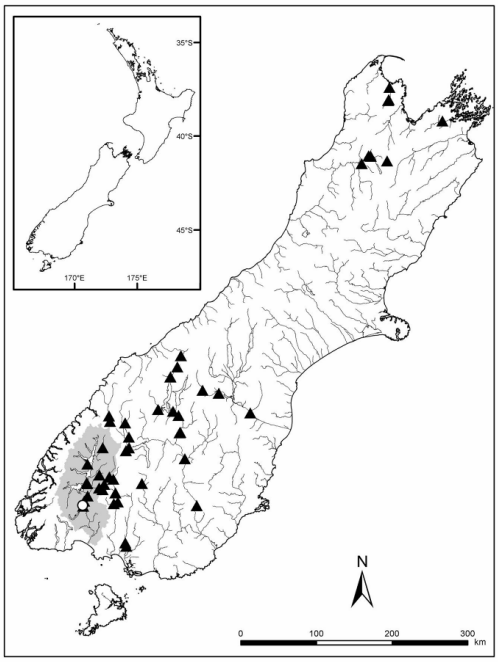

Fig. 1.2. Rivers in South Island, New Zealand which presently contain *Didymosphenia geminata*. (Map sourced from C. Kilroy.)

a) What are the consequences of the invasion?

In New Zealand, *D. geminata* is in a state of constant bloom conditions, this poses a problem for all users and uses of the rivers. First, the bloom conditions are not aesthetically pleasing for recreationists, such as fishers, swimmers and kayakers. This is due to the appearance and texture of the thick mat covering most of the river bed. When in bloom, the mats become so thick that the flow detaches pieces which float downstream and out to sea, unless they become caught on obstacles. Recreationists find floating mats to be unpleasant and a nuisance when they become trapped in sports gear.

Second, floating mats of *D. geminata* cause irrigation screens and power generation dam screens to clog (Kilroy 2004). Irrigation water pumped from infected rivers causes clogging of nozzles on farm irrigators. Each affected nozzle then has to be cleared by hand.

Third, *D. geminata* affects the ecology of rivers. Macro-invertebrates in the infected river systems are changing. Individual Plecoptera, Tricoptera, Ephemeroptera and Diptera are becoming on average smaller, and the proportion of oligochaets and molluscs are increasing (Kilroy, Biggs, Blair, Lambert, Jarvie, Dey, Robinson and Smale 2005). In New Zealand rivers rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) have been introduced for recreational fishing (Mc Dowall 1994). Trout consume invertebrates and are

also piscivorous. Macro-invertebrates are able to hide in mats of *D. geminata* more effectively than under stones. This leads to a reduction in access of invertebrate prey to fish. The reduction of invertebrate resources could cause trout to increase predation on native fish (Kilroy *et al.* 2005).

Fourth, *D. geminata* traps significant amounts of suspended sediment, which could cause a shift in sedimentation patterns. Any change in the sedimentation process could affect the build up or erosion of coastlines. Small pulses of sediment can occur due to the cyclic growth pattern of *D. geminata*. The cycle finishes when mats are thick and pull away from the substrate, releasing trapped sediment. Large pulses of sediment will occur with floods, which scour mats from the bed rock. Algal sediment storage needs to be accounted for when flood water is released from dams as part of river management. However, *D. geminata* has not been in New Zealand long enough for its full effects to be understood.

b) Current research and strategy for understanding and eradicating *D. geminata*

Biosecurity New Zealand has been proactive with funding extensive research and public awareness campaigns, including the current "Check Clean Dry" campaign (Fig.1.3). A nationwide survey found patterns of spread that suggested human-mediated dispersal, for instance, occurrence in widely separated rivers that are popular for fishing and recreation. This contrasts with the pattern of spread within catchments due to birds, livestock and other animals. The pattern of dispersal by animals would be gradual increase throughout infected rivers and slow movements upstream.

Fig. 1.3. A notice in an infected area to warn public of the risk associated with *D. geminata*.

c) Research conducted by National Institute of Water and Atmosphere (NIWA)

NIWA was commissioned in 2005 by Biosecurity New Zealand to carry out an ecological study of *D. geminata*. This looked at its environmental and aesthetic impacts on rivers and gathered additional biological information which could be useful for eradication trials in the future. The study had three components: 1) investigation of hydraulic habitat preferences; 2) short term study of temporal changes in biomass and condition in relation to hydrological changes and water chemistry; and 3) comparison of invertebrate communities from affected and unaffected sites, to assess its potential effects on higher trophic levels (Kilroy *et al.* 2005). Currently NIWA is looking at the ecological effects of *D. geminata* at different spatial scales of, run, riffle, reach, up to whole rivers.

Biosecurity New Zealand commissioned NIWA in 2006 to research the eradication of *D. geminata*. These experiments are taking place at the Monowai power station where an artificial stream system is being used to test the effects of different biocides on *D. geminata* using water from Monowai River (Jellyman, Clearwater, Biggs, Blair, Bremner, Clayton, Davies, Getz, Hickey, and Kilroy 2006). The effect of each of a range of biocides (chelated copper, zinc sulphate, pine-based organic compound, germanium dioxide, simazine, quaternary ammonium

compound, chlorine, diquat, hydrothol, and ethylenediaminetetraacetic acid) is being tested on *D. geminata*, native and introduced fish, macro-invertebrates and native algae.

There are three stages of investigation of biocides at a range of concentrations: 1) in the laboratory, 2) in an artificial stream and 3) in a natural stream. The biocides will be evaluated according to:

- toxicity to *D. geminata*;
- potential for affecting the integrity of the non-living stalk material;
- contact time;
- ease of application;
- potential for damaging key non-target species;
- ecosystem functioning;
- cost of materials and application;
- consent requirements or restrictions (Jellyman *et al.* 2006).

The research continues and is inconclusive at this stage. However, chelated copper, EDTA and Organic Interceptor™ are going to be further tested in later stages. These products are the most effective biocides when compared to the control samples (Jellyman *et al.* 2006).

1.12 Project aims

The overall aim of this study was to investigate the range of conditions in which *D. geminata* can survive and the speed at which it accrues biomass in a typical river environment. The Waitaki River was selected for the field study as it is the closest infected river to Christchurch and it has a stable flow. The results of these experiments suggest how *D. geminata* could travel to New Zealand from the Northern Hemisphere.

Questions addressed were:

- How long can *D. geminata* survive in a range of environmental conditions?
- How fast does it colonise artificial substrates in the field?
- Does the invasion of *D. geminata* fit any of the currently proposed theories of invasion?
- What are the policy implications of results obtained in this study?

Chapter two covers the field experiments in the Waitaki River. Chapter three describes the laboratory experiments used to investigate survivability. Chapter four reviews the international and national policies surrounding biosecurity issues. Chapter five discusses the implications of this project on policies and management options.

Chapter 2: Field Experiments

2.1 Introduction

The field study was conducted in the Waitaki River, which is located 18km north of Oamaru (Fig. 2.1). The Waitaki is a braided meandering river which starts in the Southern Alps and flows through a number of dams to the coastline at Oamaru. It has an average discharge from Kurow of 352.50 $m^3 s^{-1}$, which is above site four (NIWA unpublished data).

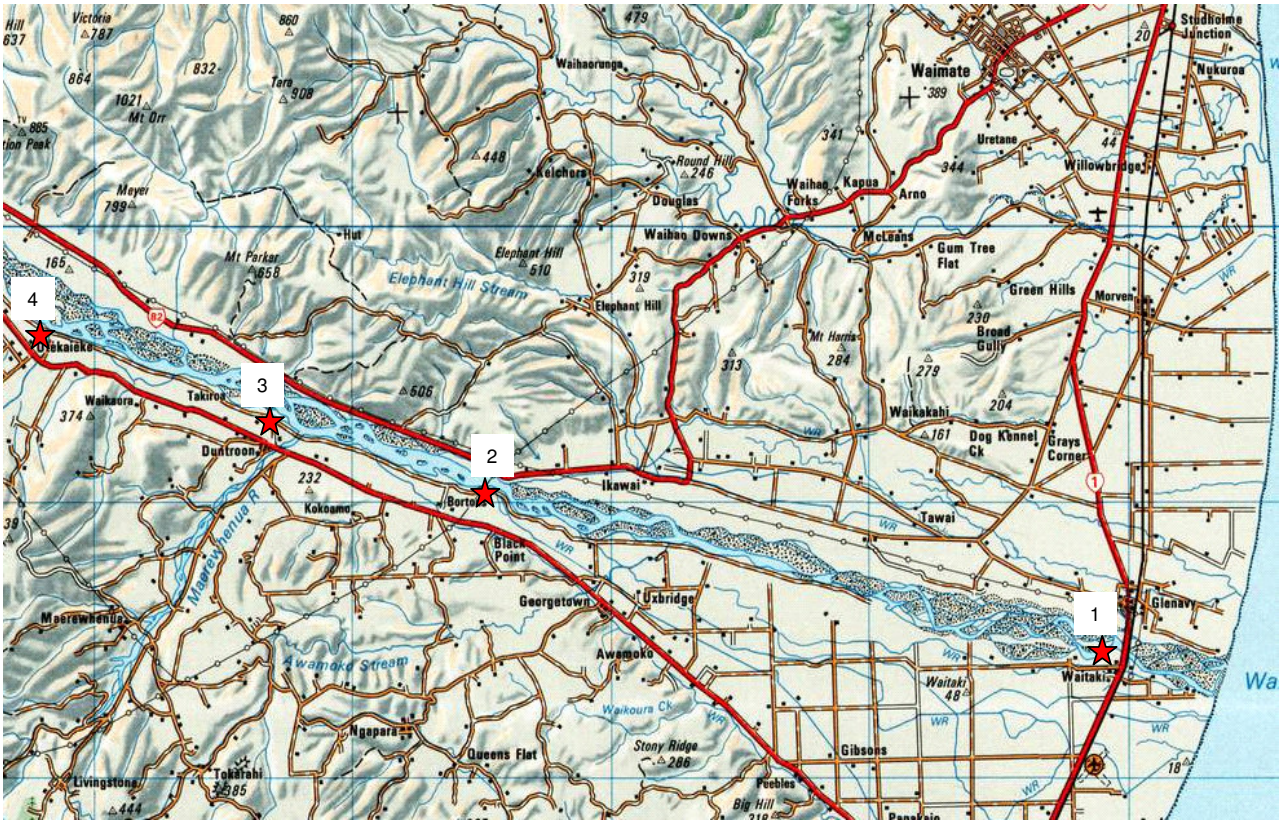

Fig. 2.1. Map of the Waitaki River from Kurow to the coast indicating all four study sites.

The Waitaki riverbed is greywacke in the form of gravel 69-85%, and sand 13-20%, which supplies an estimated bedload of 164 000 $m^3 y^{-1}$ to the coast (Hicks *et al.* 2003). Ultimately 20% of this becomes beach material. The suspended inorganic silt is variable from Kurow to State Highway 1. 95% of particles are 53 - 0.4 µm in diameter with a mean size of 4.5 µm and turbidity of 7.7 NTU (Hicks *et al.* 2003). At Kurow average pH is 7.79 and conductivity is

57.45 μS cm^{-1}. Both values have varied little from 1989 to 2003 (NIWA unpublished data). Figure 2.2 portrays a typical view of the Waitaki River at site three (Duntroon).

Fig. 2.2. Waitaki River at site three. The artificial substrates used in the experiments can be seen in the foreground as light patches.

2.2 Aim of field studies

The aim was to observe the time that it takes *D. geminata* to colonise new substrates and then to form abundant, thick mats. The initial establishment time will be dependant on the number of colonising cells in the water column. When site choice was being debated, some sites were expected to have a low cover of colonies of *D. geminata*, an indicator of low cell concentration in the water (Fig. 2.3). However, in the Waitaki at that time there were no sites with a low concentration of cells in the water column that were accessible. The sites chosen did have variability in cell density just not as extreme as planned. The Clutha and Hawea Rivers were also sampled but the flow was artificially raised and lowered frequently, preventing the use of current sampling techniques.

All chosen sites had thick mats of *D. geminata* and relatively easy access. This study investigated how water temperature affected biomass accrual, and whether densities of cells suspended in the water affected rates of colonisation.

Fig. 2.3. A rock in the Hawea River with new colonies of *D. geminata*, arrow indicates colonies, seen here as dark brown spots. Individual colonies are smaller than a 10¢ piece.

2.3 Study sites

Site one (Bridge) was at State Highway one close to the coast (Fig. 2.4., 2359935 E, 5584500 N NZMG). The site was shaded with trees growing in and around the river. Water depth was less than one metre where the artificial substrates were placed. Filamentous green algae were present with *D. geminata.*

Site two (Irrigation) was downstream of an irrigation outlet (Fig 2.5, 2335210 E, 5584500 NZMG). Water depth was less than one metre where the artificial substrates were placed. This site had full sun with no shading. *D. geminata* was the dominant species covering the sides of the river bed in a thick mat. No other algae were visible. Towards the middle of the river smaller pebbles dominated the substrate. Here there was no *D. geminata* as the substrate was easily disturbed by the flow.

Fig. 2.4. Site one (Bridge) in summer (January 13th2007) after a willow tree collapsed, the willow is in the water on the right.

Site three (Duntroon) is downstream of Duntroon boat ramp (Fig. 2.6, 2327200 E, 5592995 N NZMG). It had full sun with no shading. *D. geminata* covered the river bed in a thick mat. There were no other algae visible. The water was approximately one metre deep where the substrates were placed.

Fig. 2.5. Site two (Irrigation) looking downstream, in spring (September 15th2007) Waitaki River main braid after irrigation water has been extracted.

Fig. 2.6. Site three (Duntroon), looking downstream in mid- summer (January 27th2007) when the flow was at its highest.

Site four (Grants Road) is the closest point to the upper limit of accessible *D. geminata* (Fig. 2.7, 2316050 E, 5599160 N NZMG). The site had full sun with no shading. *D. geminata* covered the river bed in a thick mat. There were no other algae visible. This site consistently had the highest recorded velocity of all sites. The water was approximately one metre deep where the substrates were placed.

Fig. 2.7. Site four (Grants Road) looking upstream in spring (September 15th 2007). The main braid is on the right, the experimental substrates were situated close to shore due to the high water velocity.

2.3.1 Flow data

The stage height (Fig. 2.8) and flow rate (Fig 2.9) showed some variability throughout the year. Both stage height and flow rate increased over the summer period. The variability in stage height and flow rate was consistent enough for research to be undertaken from September 2006 to February 2007.

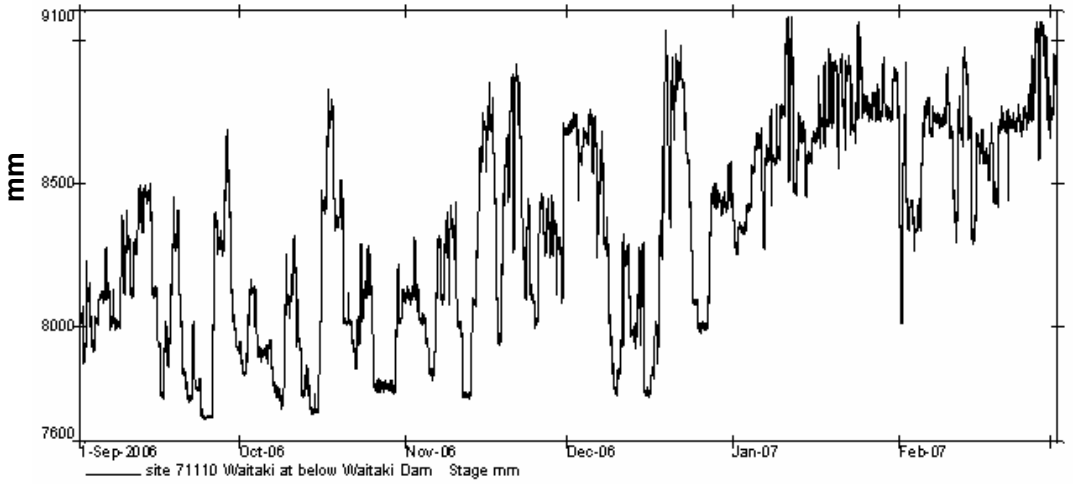

Fig. 2.8 Stage height of the Waitaki River (mm) at Kurow from September 2006 to March 2007. (Unpublished data from NIWA)

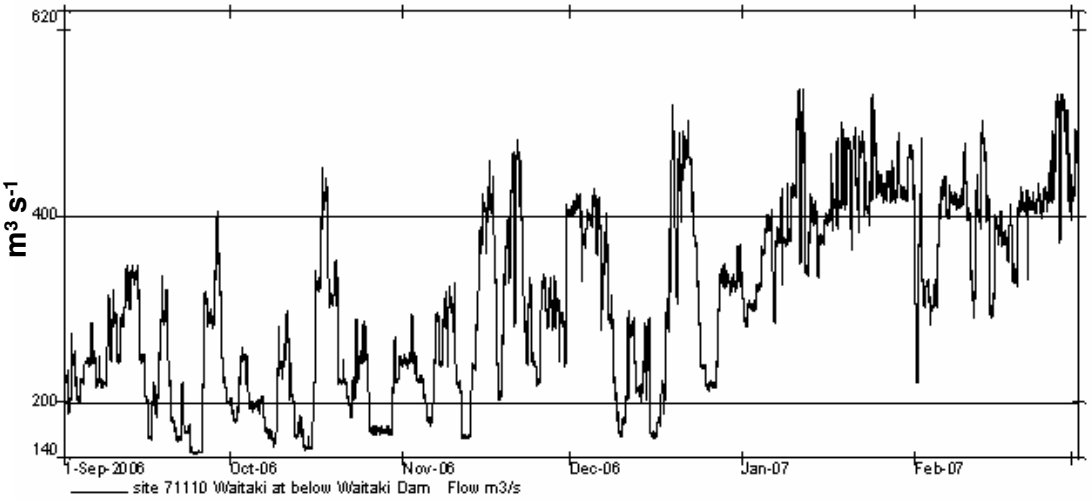

Fig. 2.9 Flow rate ($m^3 s^{-1}$) of the Waitaki River below the Waitaki Dam from September 2006 to March 2007. (Unpublished data from NIWA)

2.4 Methods

Three artificial substrates were placed at each of the four sites. Substrates were made out of blow-moulded plastic formed into sheets with individual half-bubbles with an area of

approximately 22 cm^2, glued to a sheet of durable thick plastic with a hole at each end. Substrates were then anchored to the river bed by bolting the sheet of plastic to concrete blocks (Fig.2.10).

Fig. 2.10. Artificial substrate used in Waitaki River. The top left hand corner shows the substrate bolted to a concrete block. The substrate is approximately one metre long and the concrete blocks weighed over 3 kg.

The substrates were placed one metre apart and at approximately one metre depth. Site four was always sampled first and site one last, so that sampling times of each site were reasonably consistant. Each site and substrate was photographed with a Lumix DMC-FX01 digital camera. Water velocity was measured with a velocity metre (FlowTracker handheld ADV, Acoustic Doppler Velocimetre) and temperature measured with a combined temperature / conductivity / pH meter (wp81, TPS Pty Ltd, Brisbane). A suspended solid sample was taken with a 40 µm mesh net with a 120 ml screw-capped container (Labserv) attached to the end, for two minutes downstream of each substrate. *D. geminata* cells and mat fragments and other detritus were caught in the net. Once the suspensiods were caught in the net, water was scooped into the net to flush the detritus to the container. Water that is held in the container was displaced by the suspensoids (Fig. 2.11).

At each subsequent sampling, the substrates were taken out of the river and one bubble of plastic removed. The bubble was placed into a small sterile plastic bag (Whirl Pak, Nasco) which was sealed. At each site the temperature and water velocity were recorded between 13:00 to 16:00 h. Photographs were taken of the substrate at each location. A suspended solid sample was taken with the 40 µm mesh net, for two minutes downstream of the substrates.

The detritus was placed in polystyrene 120 ml screw-capped containers. Clumps of *D. geminata* floating downstream (Fig. 2.12) were often caught by the net. Sampling occurred every other week in spring from September 15th 2006 to October 29th 2006, and in summer December 30th 2006 to February 25th 2007. All samples were taken back to the laboratory and frozen until required for laboratory analysis.

Fig. 2.11 Net with container on the end, used to capture material suspended in the water flow.

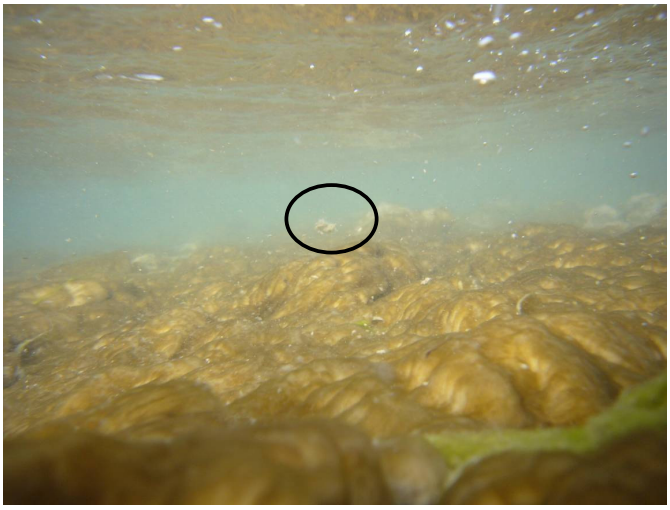

Fig. 2.12 Thick mat of *D. geminata* at site three (Duntroon September 15th 2007). In the centre of the image is a suspended clump of *D. geminata.* Clumps such as this were often caught in the mesh net.

In the laboratory each bubble of plastic was brushed with a toothbrush to clean off all algae and then washed with water. The water was then filtered out of the sample. The sample was

frozen, freeze-dried, weighed and put into a furnace at 400°C for four hours. The sample was then reweighed in order to obtain the ash free dry weight (AFDW). The suspended solid sample was defrosted and 1 ml was placed in a microscope well and viewed at 100 x magnification using an inverted microscope. A count was made of all *D. geminata* cells within the well. Three sub-samples were counted from each net sample. When cells covered the entire well, there were too many to count, so three random fields of view were counted instead of the entire well.

2.5 Results

2.5.1 Temperature

Temperature increased over the sampling period (Fig 2.13) which was expected as the second set of sampling occurred over summer. There was little difference between sites, except on January 13th when two sites were 4°C warmer. Average temperatures were consistent with NIWA data which has been recorded monthly at Kurow since 1989.

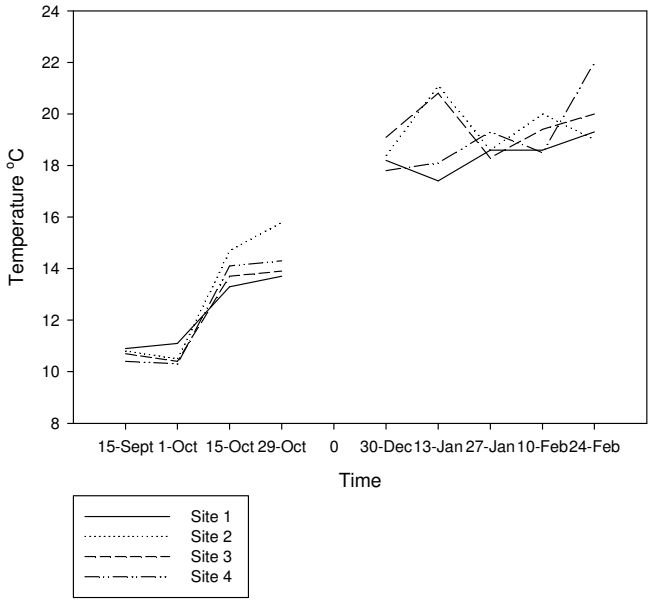

Fig. 2.13. Temperature of Waitaki River at the four sites over the periods of sampling.
There is a warming trend from spring to summer. The gap in the graph represents a period during which no samples were taken.

3.5.2 Abundance of cells suspended in river water

The number of cells ranged on average from 100-200 cells L^{-1}. The initial establishment probably occurred in the first day or so. Therefore initial establishment was never documented

as it occurred too quickly. There is variability in cell density over time and between sites (Fig. 2.14).

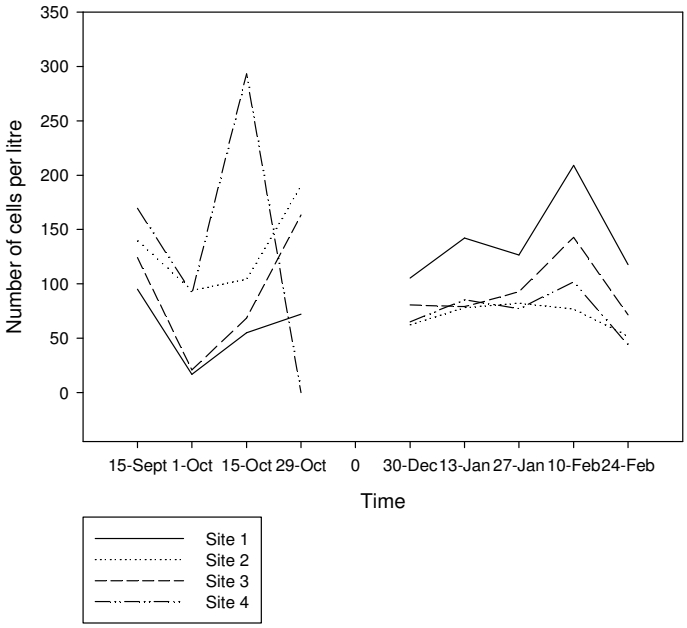

Fig. 2.14 Cell density of *D. geminata* in the river water over spring and summer. In summer all sites had similar cell densities. In spring, site four had markedly different densities on two sampling occasions.

3.5.3 Ash free dry weight of substrate samples

The ash free dry weight (AFDW) was used as a measure of biomass. In spring there was a gradual increase (Fig. 2.15) and only at site three did biomass accrue to levels similar to those of summer. During summer the biomass at site three was much higher than all other sites. After six weeks in summer, AFDW was 5500 mg, compared to sites two and four which had AFDW of 250 mg. The average weight of *D. geminata* (kg m^{-2}) over spring and summer shows that it has become an ecosystem dominant in the Waitaki River (Table 2.1).

Table 2.1 The average kg m^{-2} of *D. geminata* over summer and spring sampling

	Average kg m^{-2}	
	Spring	Summer
Site 1	0.035025	0.001185
Site 2	0.025814	0.068878
Site 3	0.137569	1.047893
Site 4	0.032942	0.122699

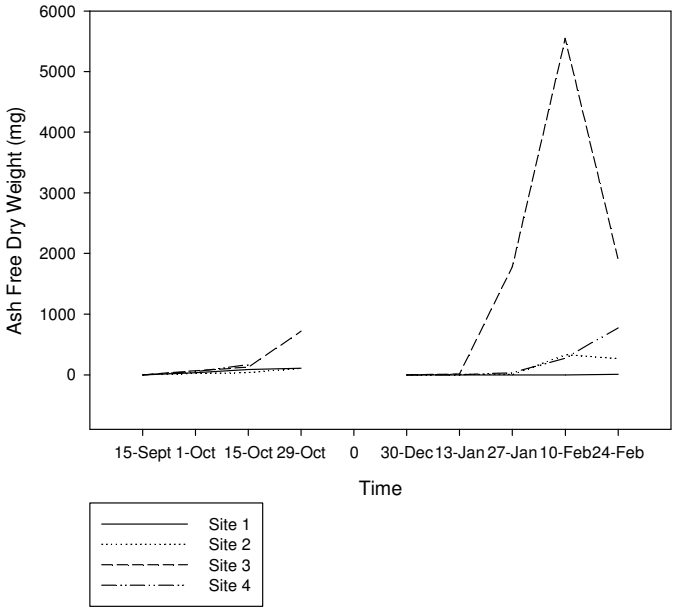

Fig. 2.15. Ash free dry weight (AFDW) of *D. geminata* from the artificial substrates at each site throughout spring and summer. Site three had unexpected biomass growth during summer. Site four was sampled only twice during spring as the substrates were washed away down stream.

Figure 2.16 illustrates how rapidly *D. geminata* can grow in spring at site four in the Waitaki River, the photographs represent one month of growth. Figure 2.17 demonstrates the extent of growth of *D. geminata* at site three over the short period of summer sampling, and illustrates the thickness of the mat where the bubbles of plastic are completely obscured.

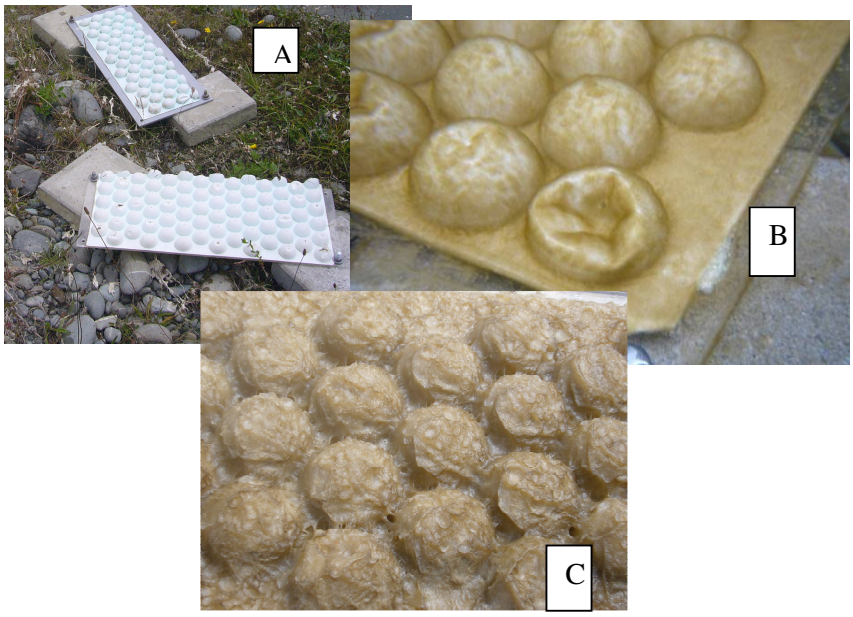

Fig. 2.16 Substrates at site four over winter, A) substrates before entering the river on September 15th 2006, B) substrate on October 1st 2006, and C) substrate on October 15th 2006. These photographs demonstrate how rapid *D. geminata* can grow.

Fig. 2.17. Artificial substrate in summer (February 25th 2007) after eight weeks placement at site three. The bubbles of plastic have been totally covered with *D. geminata*.

2.5.4 Inorganic mineral content of substrate samples

The ashing process provided an estimate of the inorganic mineral content of each sample, which is mostly comprised of sand. Increase in sediment over time was expected (Fig. 2.18), this pattern is caused by increased length of the diatom stalk, which enables it to trap more sediment. River flow drags on the stalks and eventually pulls the mats free of the substrate. This then causes the sediment to be released downstream either in suspension or inside dislodged mat material. Site three held the most sediment as the length of the stalk was longest.

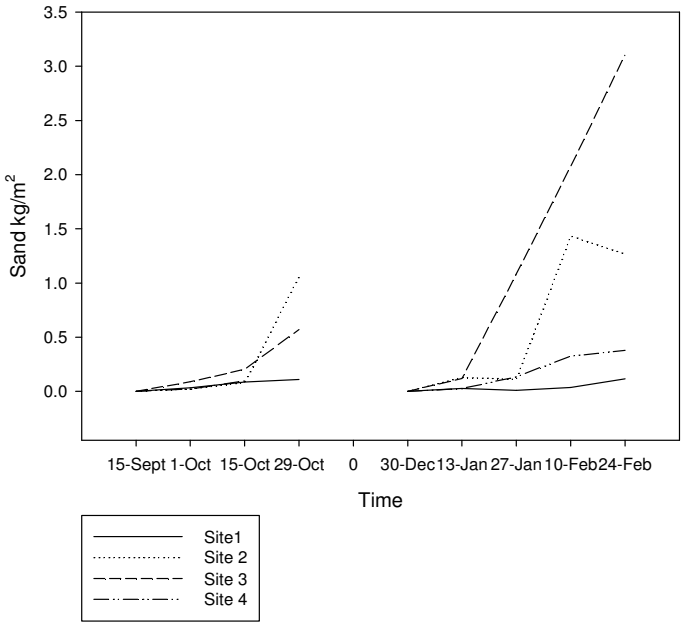

Fig. 2.18 Inorganic mineral content of substrate sample. This comprised mostly sand (pers. obs.). Most samples held less than 1 kg m^{-2}. Site three had an average of 1.7 kg m^{-2} and a maximum of 5.23 kg m^{-2}.

There was a considerable amount of sand being trapped by *D. geminata* in Waitaki River throughout the year. It was especially evident during summer with an average volume of 13,560.5 kg m^{-3} over 25kms of the Waitaki River. Site three samples contained the most sand (Table 2.2).

Table 2.2. Sand trapped by *D. geminata* over a 25 km stretch of Waitaki River. The totals were calculated as follows; average kg m^{-2} (taken from AFDW) x length of the river (2500m) x width of the river(80m) x height of *D. geminata* (0.02m) /4. The number was divided by four as *D. geminata* is generally at the edges of the river and covers an estimated 25% of the total area of the riverbed.

	Average kg m^{-2}	Total kg m^{-3}	Elapsed time
Summer	0.733	13 560.5	8 weeks
Spring	0.176	3 256	6 weeks

The force of the river peeled the *D. geminata* mat from the surface of the substrate due to the extreme length of the stalks (Fig. 2.19). This revealed the sand being trapped by the mats. The dark brown surface of mat is caused by the presence of numerous live cells whilst pale brown is the stalk and associated sand. If the sand was centrifuged out of the stalks they would look white.

Fig. 2.19 *D. geminata* is pulling away from the artificial substrate after eight weeks at site three (February 25th 2007).

2.5.5 Interactions amongst variables

Figures 2.20- 2.22 compare individual variables with each other to find patterns. Patterns of AFDW and cell density in the water column do not match well over winter but correlate better during the summer months (Fig.2.20).

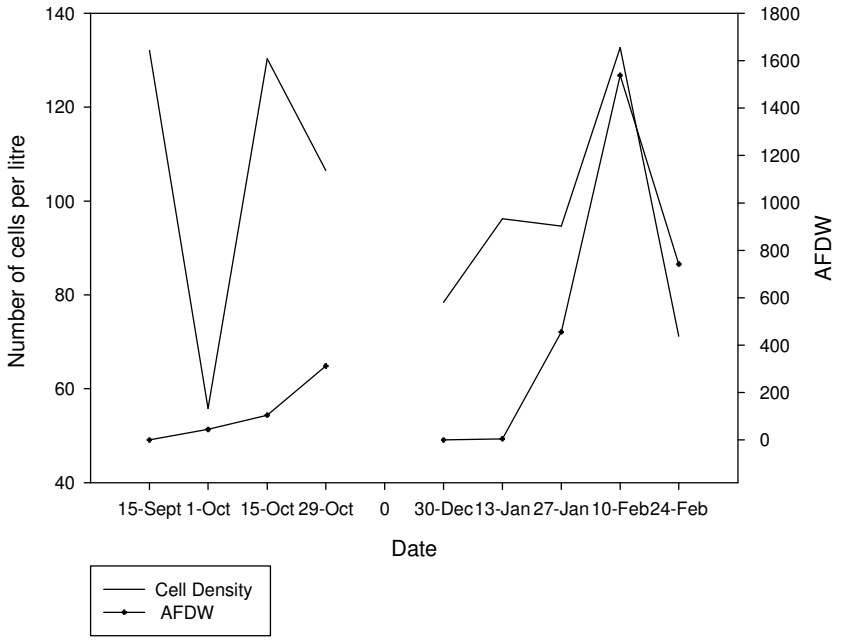

Fig. 2.20. AFDW and cell density over both sampling periods. The winter AFDW is not at all correlated with cell density in the water. In summer the cell density correlates closely with AFDW.

Biomass accumulation of *D. geminata* correlated well with its sediment holding capacity (Fig. 2.21). AFDW was reduced in the last sample in summer but still held significant sand. *D. geminata* is a cold water diatom that has optimum growth between 10 -17°C (see chapter three). Biomass during spring increased slowly, until the water temperature rose to 12°C, then the biomass increased exponentially (Fig. 2.22). However, during summer, biomass increased exponentially immediately, even though daytime temperatures were 18-20°C.

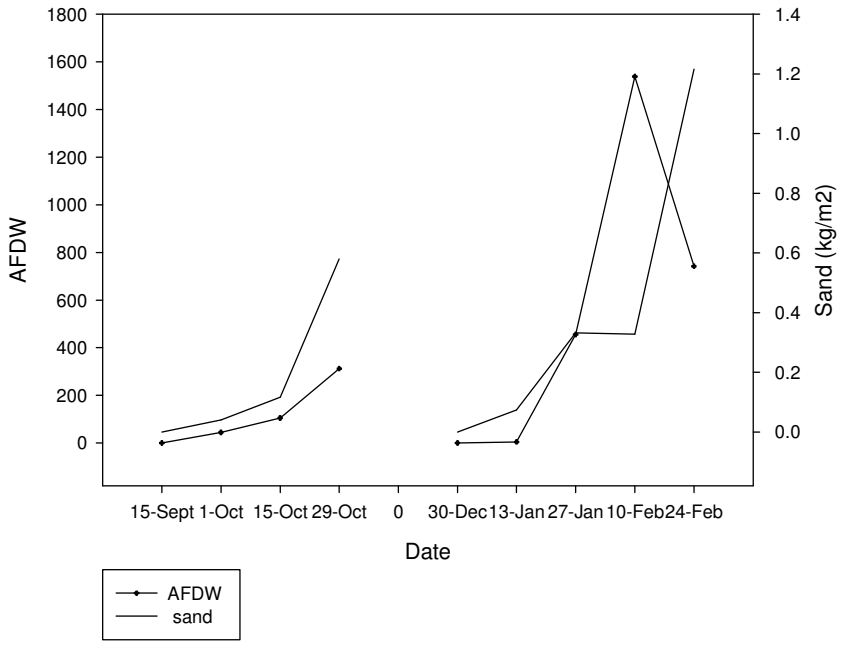

Fig. 2.21. Quantities of AFDW and trapped sand over time showing their close correlation, both AFDW and sand require time to accumulate weight.

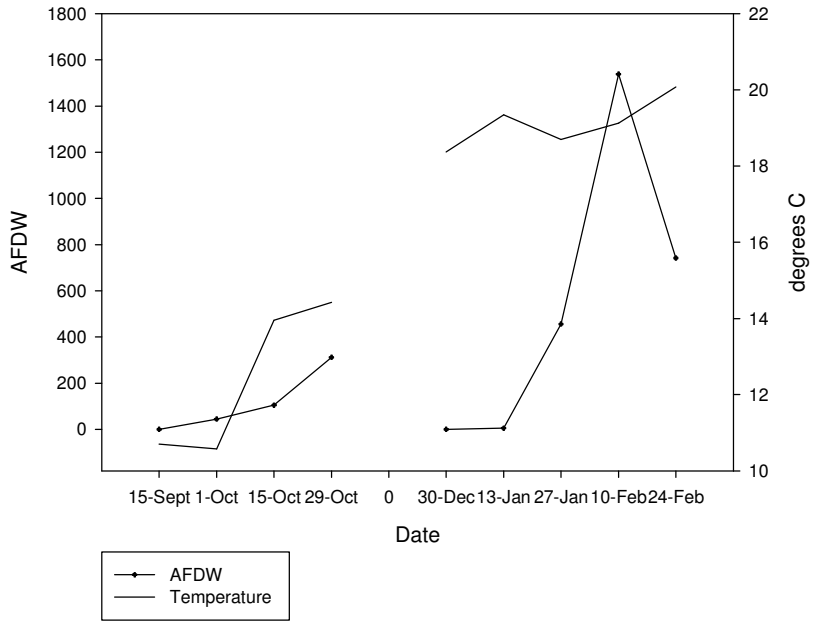

Fig. 2.22. Changes in temperature and biomass accrual (as AFDW) with time. With increased temperature over summer, biomass accrued quickly. Laboratory tests (chapter three) showed that 10-17°C is the optimum temperature for this diatom.

2.6 Discussion

2.6.1 Temperature

Temperature of the river affected the timing of growth and the amount of growth. In summer, warmer temperatures induced quick growth, even though the temperatures had previously been thought to be too warm for *D. geminata* to survive for long periods. However, it is possible that a drop in temperature overnight could lessen the stress on developing colonies. Results suggest that until water temperature exceeds 12 °C the accumulation of biomass is slow (Fig2.22).

The optimum seasons for this diatom are spring through to late autumn, although some die back will occur with consistent high temperatures. Therefore eradication efforts should be in mid-winter when *D. geminata* is growing the slowest.

3.6.2 Cell density in the water column

The expected pattern for cell density is as follows: the higher the cell density in the water column the faster biomass should accrue at the initial establishment phase. However, when the water column has a high abundance of suspended cells available for colonisation of stone surfaces then the establishment time is then unaffected. In fact, this study found that the critical time for establishment then becomes the first few hours to days, not weeks.

3.6.3 Ash free dry weight

The biomass accumulation at site three over summer was unexpected. By the second sampling during summer, biomass was already considerably greater than other sites. Due to the design of the substrate, there were technical difficulties at some sites, which will have contributed to some of the low AFDW figures. These difficulties include substrates being consistently overturned, and large clumps of *D. geminata* becoming caught on the substrate and shading the substrate in patches (Fig 2.23).

A number of substrates were washed away at various times by high river flows. In spring, samples were taken on only four occasions, as too many substrates had been dislodged. Sampling was prevented at site two during the summer sampling when the river level increased and the water was too deep and swift to remove samples.

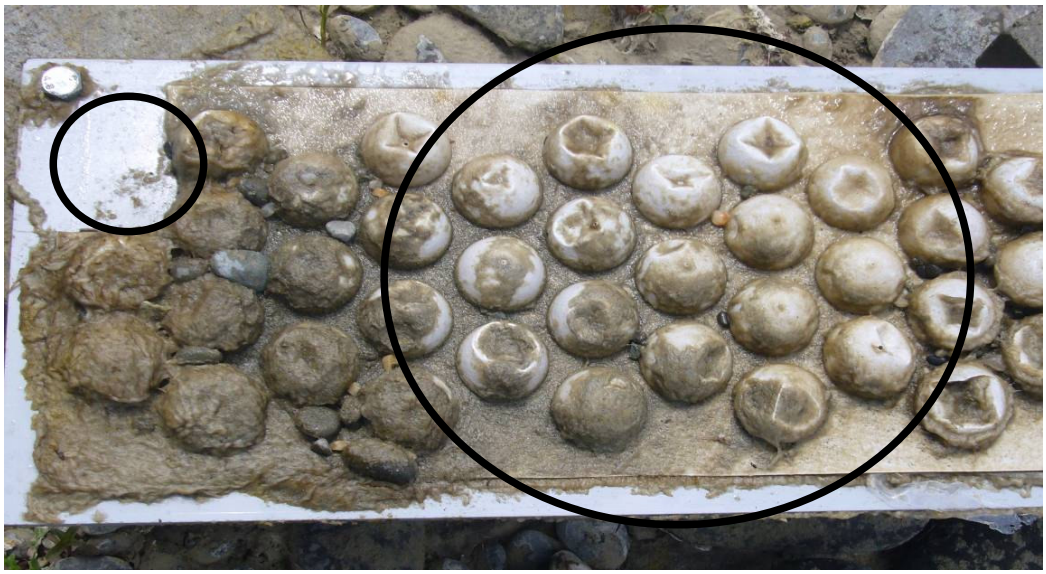

Fig. 2.23 The artificial substrate at site two after a suspended portion of *D. geminata* mat had settled onto the substrate. *D. geminata* has not grown where it has been shaded by the settled mat seen inside the large circle. The smaller circle shows where some samples have been taken from the substrate.

At site one, which was the only site that had shade and competition from other algae, *D. geminata* was very dark in colour and had short stalks (Fig. 2.24). This suggests that in shaded conditions it increased the number of cells without increasing the length of stalk.

Fig. 2.24 Substrate at site one (October 29th 2006), two weeks after being deposited in the river, the substrate been shaded for this period. The *D. geminata* is very short and very dark brown in appearance.

2.6.4 Sediment

The amount of sand being collected and stored in the mats of *D. geminata* could have consequences for the coastline. As the coastline around the mouth of Waitaki River is already eroding, this reduction in sand could increase the erosion. The importance of algal sediment storage needs further study.

2.7 Conclusions

D. geminata has invaded New Zealand's southern rivers, causing widespread consequences. Where, the water quality, substrate material, velocity and temperature are favourable to its exponential growth. This field study shows that *D. geminata* is growing in a greater range of temperature and light conditions than previously recognised. Sediment storage has also become a factor for river management.

Chapter 3: Survivability of *Didymosphenia geminata*

3.1 Introduction

Didymosphenia geminata (Fig. 3.1) is a freshwater alga, and specifically a diatom (class Bacillariophyceae, phylum Ochrophyta). Diatoms have siliceous cell walls which are symmetrical and usually intricately patterned. The walls have two main components called valves that fit together like the base and lid of a box. The whole wall structure is termed a frustule (Kilroy 2004). The patterns on the valves consist of small pores which form rows termed striae. *D. geminata* has large cells >100µm long and 40 µm wide (Kilroy 2004) which are individually attached to the end of branched mucilaginous stalks that attach to a substrate (Round *et al.* 1990). *D. geminata* can be epiphytic (attached to plants) and epilithic (attached to rocks) (Round, Crawford and Mann 1990). The *D. geminata* populations found in the lower Waiau in 2004 were of the monotype *capitata*, commonly found in northern Europe (Kilroy 2004).

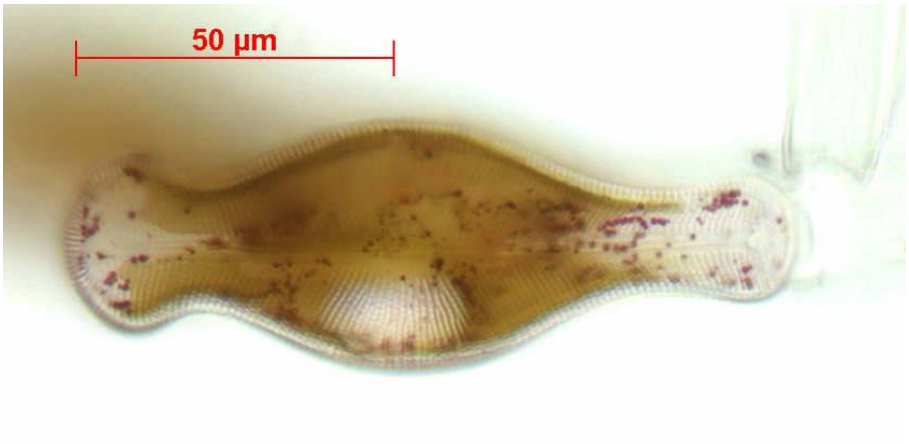

Fig. 3.1 *D. geminata* cell stained with neutral red. This cell is alive and healthy. The brown pigmented structure within the cell is the chloroplast. The mucilaginous stalk would attach to the right-hand apex of the cell. The striae are visible, covering the valve. The striae are made up of rows of small pores which can just be resolved in this image.

D. geminata reproduces asexually by binary cell division, as do all diatoms. A valve of the original cell is retained by each daughter cell whilst a new, smaller valve is formed that fits inside the original one. The slight size difference between the two valves means that repeated cell division results in a gradual reduction in average cell size of the population. Most diatoms undergo sexual reproduction at some point to restore the size of the population. This has yet to

be observed globally in *D. Geminata.* Individual cells reproduce to form small colonies, colonies then merge together to form brown mats which can be very extensive.

There are numerous vectors for *D. geminata* dispersal within a catchment and between catchments; these vectors include birds and livestock as well as fishers and kayakers (Kilroy *et al.* 2006). Knowledge of survival limitations could increase the possibility of predicting the viability of cells attached to feathers, fur, the hooves of stock animals and human vectors such as kayaks, fishing equipment and car wheels.

The following laboratory experiments investigated the survival limits of *D. geminata* at different levels and combinations of temperature, light and moisture. The combinations used were all relevant to conditions which would be experienced during dispersal. Earlier experiments found it was difficult to maintain *D. geminata* colonies collected from the natural environment for long enough to complete experiments in the laboratory (Kilroy *et al.* 2006). Therefore survival times were hypothesised to be short.

Hypotheses tested were:

1. Survival will be of greater duration at 12 and 5 ºC, at higher light intensities.
2. Survival will be of shorter duration at 28, 20 ºC, irrespective of light intensities.
3. Survival will be greater in wet conditions compared to those in damp conditions.

3.2 Methods

Rocks (approximatley 10 cm in diameter) supporting growths of *D. geminata* were collected from Waitaki River, between Kurow and Duntroon. They were placed in household plastic containers filled with river water, chilled on ice in chilly bins for transportation to the NIWA laboratories at Riccarton, Christchurch, where the experiment was undertaken. The laboratories include a MAF-approved controlled environment with a Contherm Phytotron Climate Simulator equipped with 12 x 36W fluorescent tubes. Lights were set on a 16 hour light, 8 hour dark regime.

In the laboratory, *D. geminata* mats were scraped off the rocks and cut into 10 x 10 x 5 mm pieces, ensuring each piece contained an area of healthy brown cells, and placed into 35 mm diameter Petri dishes. Pieces in Petri dishes will be termed samples. The Petri dishes were labelled, placed in their respective trays and put in the incubator at the desired temperature. Each tray had another tray of the same type taped to the top as a lid. The high light treatment had clear trays, the dark treatments had black trays and the medium light condition had clear trays placed in a white cloth bag. Low light was achieved by placing clear trays in a dark blue cloth bag. Each tray had 24 Petri dishes.

3.2.1 Temperature treatments

Temperature treatments were: 28, 20, 12 and 5 ºC. Incubator thermostat settings were maintained within 5 ºC of the target temperature. The temperature was logged every 30 minutes using a Hobo tidbit logger.

3.2.2 Light treatments

At 28 and 20ºC there were three light treatments: high, medium and dark. An additional low light treatment was added at 12 and 5 ºC, as there was little difference in the high and medium light treatments at 20ºC (Table 3.1). Light levels within the trays were measured in the simulator (lights on) using a LiCor light meter. Treatments were as follows;

- High light (65 µmol m^{-2} s^{-1});
- Medium light (34 µmol m^{-2} s^{-1});
- Low light (4 µmol m^{-2} s^{-1});
- Dark (0 µmol m^{-2} s^{-1}).

Table 3.1. The light treatments at four temperature conditions.

ºC	High	Medium	Low	Dark
28	**X**	**X**		**X**
20	**X**	**X**		**X**
12	**X**	**X**	**X**	**X**
5	**X**	**X**	**X**	**X**

Compared to natural light the experimental intensities were dim. Full sunlight is approximately 1500 µmol m^{-2} s^{-1}. However, these intensities are typical of those of laboratory experiments (Kilroy *et al.* 2006).

3.2.3 Moisture treatments

Of the 24 Petri dishes in each tray, twelve contained damp samples with no additional water. These samples often dried over time and became white and dry to the touch. Twelve samples were maintained in a wet condition, the dish being refilled as necessary with filtered river water.

3.2.4 Time intervals

One sample of *D. geminata* was tested at the start of the experiment to obtain an estimate of cell viability. One sample from each treatment was randomly selected and tested at 6, 12, 24, 48 h, 4 d, 6 d and then every two days until all samples were dead. After the 20 ºC trial it was clear that *D. geminata* would decline minimally over the first two days. Therefore the 12 and 5 ºC treatments were first sampled 24 - 48 h after commencement. Higher than expected survival rates meant that the sampling interval was stretched to 7-10 days or more towards the end of the trial. Each sample was destroyed in testing and therefore it was possible to run out of samples over a long period.

3.2.5 Determination of cell viability

A viability stain was used to assess the proportion of live to dead cells. A sample from each treatment was randomly selected from each treatment to be counted. The sample was immersed in Neutral Red stain (200 mg of stain dissolved in 200 ml distilled water to make a 1% w/v stock solution, further diluted to 0.05 %). Each sample was immersed for 15 minutes in 20 ml of 0.05 % solution (Kilroy *et al.* 2006). Samples were roughly macerated using scissors or rough shaking to ensure stain penetration.

A sub-sample was mounted on a microscope slide and at least 100 cells were assessed for viability. The proportion of live cells was calculated as a percentage of the total. Live cells take up the stain into their lysosomes, which become visible as small spheres of red stain (Crippen and Perrier 1974, see Kilroy *et al.* 2006 for evaluation of neutral red staining technique) (Fig. 3.2).

At the conclusion of the trials an additional experiment was conducted to check the survival of *D. geminata* in the dark treatments. Diatoms can survive following exposure to darkness for three months without changes in cell physiology and once illuminated again are able to photosynthesise (Peters 1996). Peters suggests that there are species specific differences,

however the three species that he tested were able to survive for 21 days without a reduction in growth potential and mass mortality occurred after 49 days. In this experiment samples were taken from the 12°C, wet/dark treatment and placed in full light for one week. There were nine individual samples tested, three replicates from three different rocks.

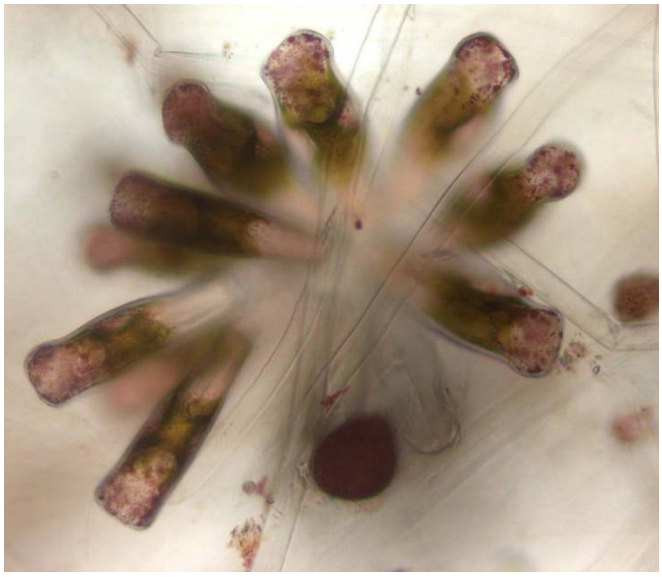

Fig. 3.2. Bundle of live cells stained with neutral red. The cells often form this pattern under the microscope if they are still attached to their stalks.

3.2.6 Statistical analysis

Logistic regression was used for the analysis, where the response variable (y) is the percentage of live cells and the independent variables are time (h), temperature (^{o}C), light level (light, medium, dark, blue shade/low light) and 'wetness' (damp, wet). The model equation included an intercept, the independent variables and their two, three and four way interactions.

3.3 Results

3.3.1 Results of tests at 28°C

D. geminata survived at 28°C for 20 - 60 h. The dark replicates died in the same time as the light replicates (Fig 3.3). The wet replicates died at the same time as the damp replicates. There is a sharp decrease in viability of all replicates between 20h - 30h. Temperature is the most influential condition at 28°C.

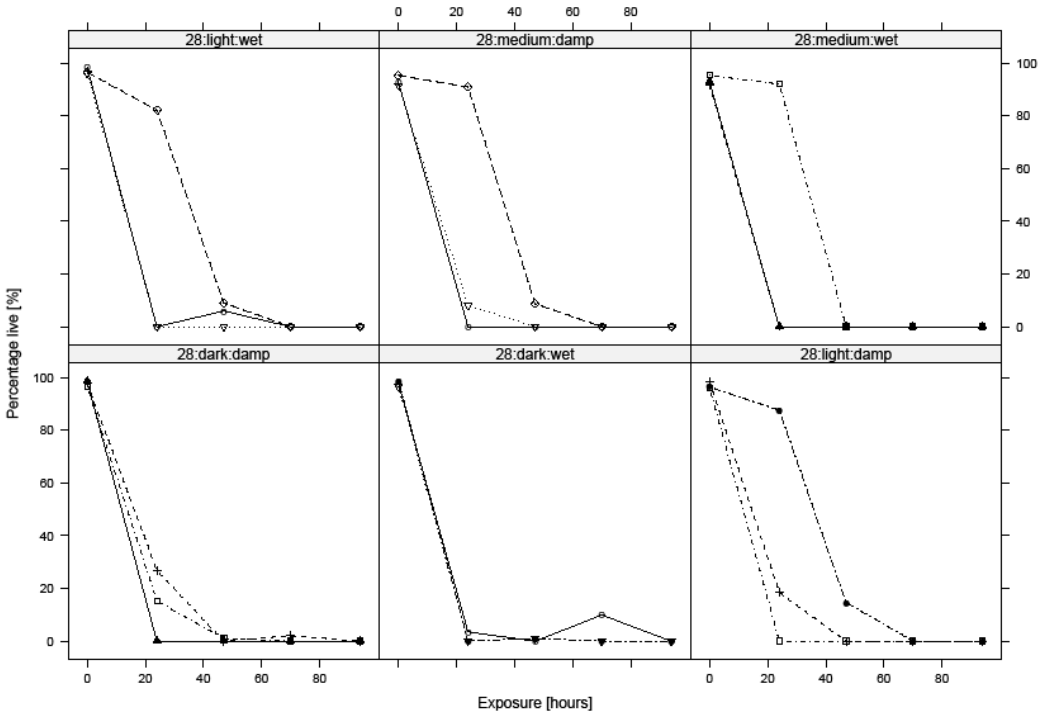

Fig.3.3. Survivability at 28°C. Each line in the graphs represents a replicate in the treatment. *D. geminata* survived for up to 60 h and all treatments showed a large decrease in viability beyond about 20h - 30 h. Light and moisture had little effect on survivability at this temperature.

3.3.2 Results of tests at 20°C

At this temperature *D. geminata* samples were able to survive up to 800 h (Fig.3.4). In the dark treatments, cells in the majority of dishes had died by 200 h although one replicate lived past 400 h before dying. The damp treatments all declined rapidly after 200h. The light treatments survived to the conclusion of the experiments. The high light condition survived better than the medium light under wet conditions (Fig.3.4).

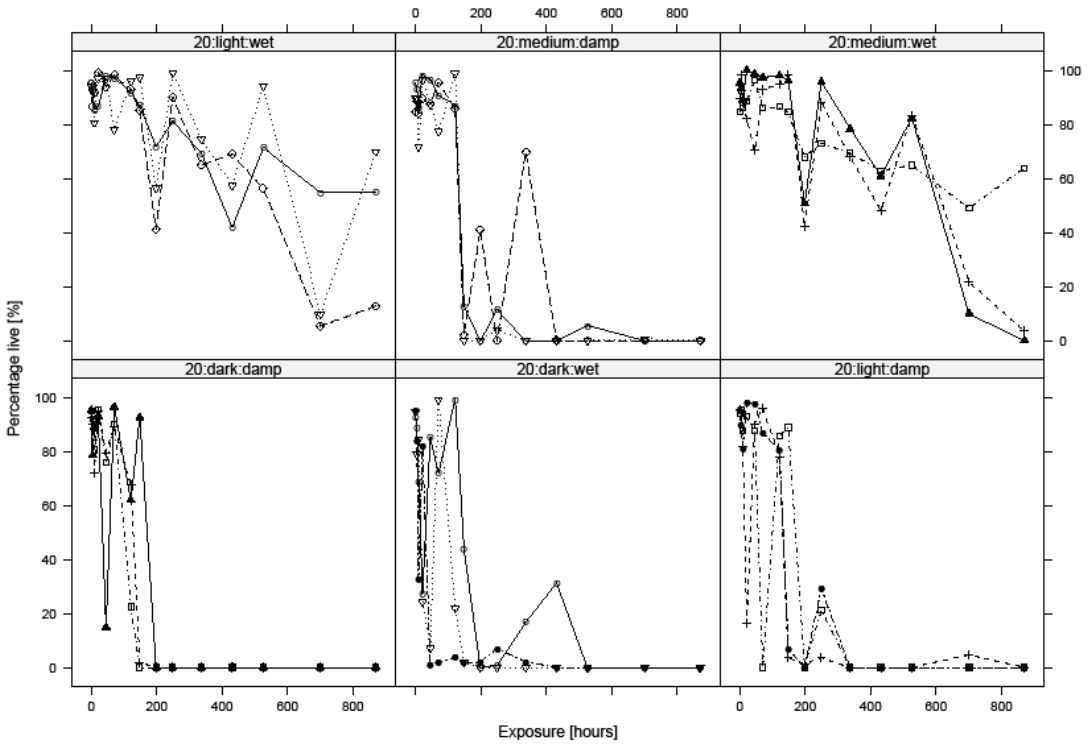

Fig. 3.4 *D. geminata* survived at 20°C over 800 h. The each treatment had different affects on survival rates. Damp treatments declined rapidly after 200h.

The damp treatment had dried with most cells dead by 300-400 h. One replicate had a few live cells at 700 h. The wet treatments survived longer than the damp treatments, up to 70% alive at the conclusion of the experiment (Fig.3.4). The variability of individual replicates within the same condition is illustrated in Figure 3.4. The experiment terminated when all samples had been used for viability testing, this was due to survival lengths well beyond prediction.

3.3.3 Results of tests at 12°C

D. geminata survived for 1500 h at this temperature (Fig 3.5), which is well beyond any predictions based on previous research. The dark treatments died before the light treatments. However they survived up to 1000 h at this temperature. The light/wet treatments survived until the conclusion of the experiment at 1500 h with 60% of cells still alive (Fig 3.5). In this treatment there is little variability between replicates. The results at low light (blue shade) differed little from those of the medium light treatment. Both treatments were variable between replicates, and some replicates survived to completion (Fig 3.5).

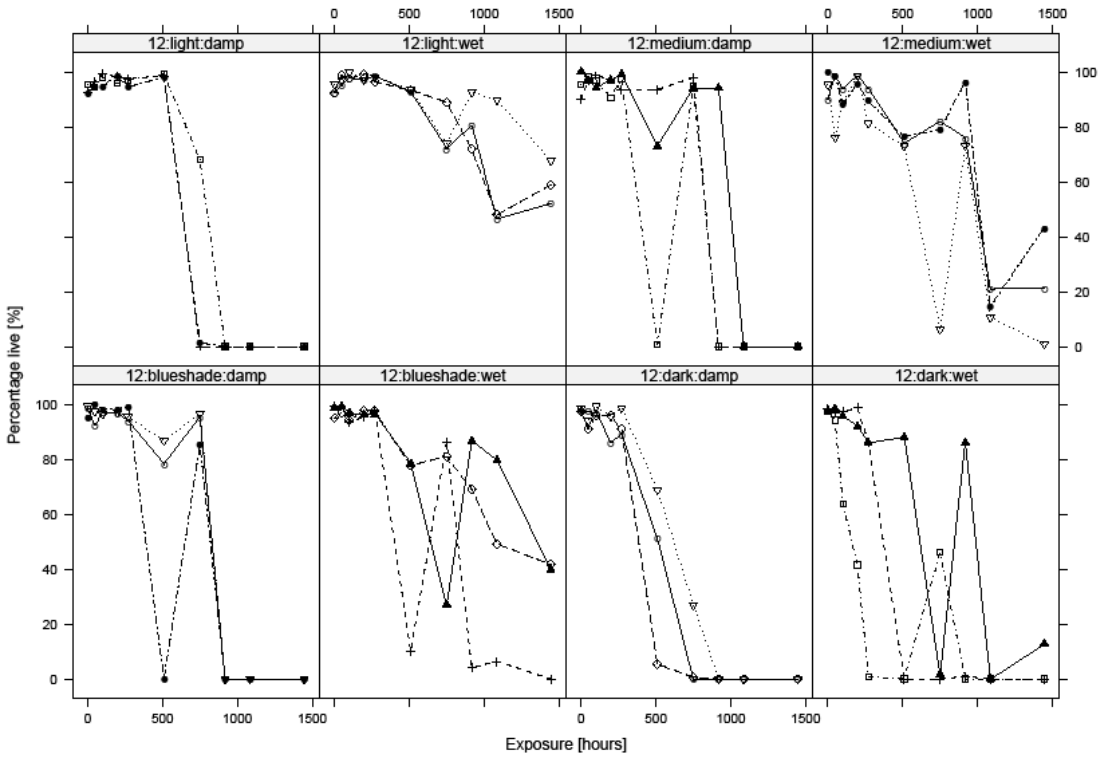

Fig. 3.5 The 12°C trial lasted 1500 hours. Both moisture level and light level contributed to the survivability of *D. geminata* at this temperature. There was some variability between replicates within treatments.

The damp treatments all dried and died before the end of the experiment between 600 and 1000 h. *D. geminata* was able to survive in the wet treatments to the conclusion. This lower temperature caused the damp treatments to survive twice as long as the 20°C treatments (Fig 3.5).

D. geminata managed to start growing in the wet treatments, which has not been reported previously. This was detected when the percent live counts started to increase and there were new brown growths visible in the dishes. Growth of this diatom in laboratory conditions requires further research. The experiment concluded when all samples had been used for testing viability. Replicates varied within treatments, because the dishes were picked from the tray and counted at random. Not all Petri dishes survived the same length of time, even though they came from the same rock.

3.3.4 Results of tests at 5°C

The dark and damp treatments survived for a longer period at the lower temperature of 5 °C than they did in any of the higher temperature treatments. At the conclusion of the experiments some treatments had 80 % cells alive at 1500 h (Fig 3.6).

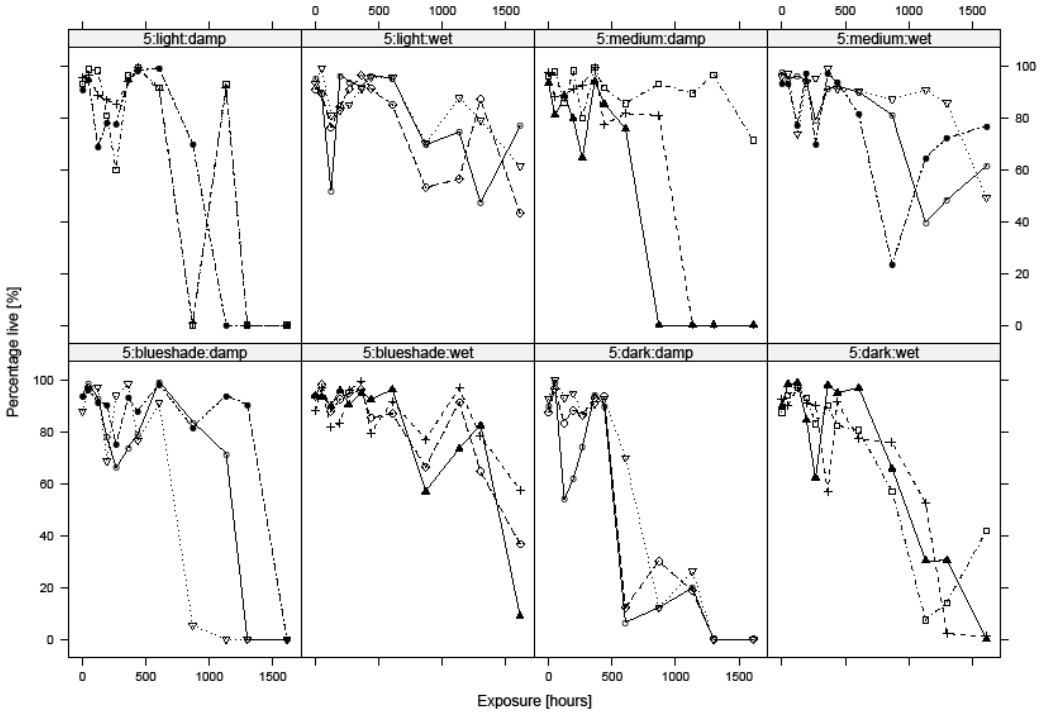

Fig. 3.6 *D. geminata* survived for over 1500 hours at 5 °C. At this temperature all treatments except light/wet had increased survival time. Additionally the replicates that survived to completion had a higher number of cells alive, compared to the 12°C experiments.

At this temperature the amount of light seemed to become less important. The dark treatments survived nearly to the conclusion. The light treatments survived to the end of the experiment, one replicate in each of the medium/wet and light/wet treatments had 80% cells alive at the termination (Fig 3.6). In the damp conditions most replicates were dead by the 1500 hours, except one replicate in the medium/damp treatment which by 1500 hours was still approximatley 70% alive. All the wet treatments survived to 1500 hours except two replicates in the dark/wet treatment (Fig 3.6).

Again cells began to grow back which is seen clearly in the medium/wet treatment. There was much less variation between replicates of each treatment compared to the 12°C experiment. The experiment concluded when all samples had been used for testing viability.

3.3.5 Cell revival tests

Revival occurred in this experiment after 60 days in the dark and one week in full light at 12 °C. Seven out of nine samples revived, three samples had 2-4% viability, two samples had around 20% viability and two samples had remarkable 61-77% viability (Table 3.1).

Table 3.2 Percentage survival of *D. geminata* cells after two months in the dark and one week in the light to revive the cells. Seven out of nine replicate samples contained cells which took up stain.

Stained	Unstained	Empty	% Viability	Replicates
2	8	68	2.56	1
35	2	135	20.35	2
25	7	74	23.58	3
0	33	118	0.00	4
0	5	85	0.00	5
2	21	79	1.96	6
118	3	32	77.12	7
3	4	78	3.53	8
85	17	37	61.15	9

Diatoms can endure darkness in a physiological resting stage (Peters 1987, Peters and Thomas 1996). Peters (1987) found a contraction in the volume of the protoplast, which was rapidly reversed when exposed to light. This response could explain the abnormal looking cells seen in some of the revived replicates (Fig.3.7).

Fig.3.7. Cells in a resting stage, protoplasts have bunched in the middle. There is some neutral red staining seen as tiny granules at the apices of the cell.

3.3.6 Analysis

All the terms in this experiment; light, moisture, temperature and time are significant (0.999 Confidence Interval). The proportion of viable cells declined over time, the rate of decline was dependent on other variables. Each variable was tested against each other to determine interactions. All the second level interactions were significant (0.95 CI), time /light, and temperature/ light. Light level/moisture was significant (0.99 CI), time/moisture, time/temperature, and temperature/moisture were all significant (0.999 CI). This suggests that the variables all interact to affect the survivability of *D. geminata*. The third level test time/temperature/moisture was also significant (0.999 CI). This test suggests that light level was not as important as the other variables. Complete results of statistical analysis are shown in Appendix two.

Figures 3.8-3.10 show the mean survival times of *D. geminata* with different variables, each variable is irrespective of the others. Low light has the highest mean survival in the light experiments, however this will be skewed as the low light was only present in two trials not four (Fig. 3.8); low light would have similar mean survival as the high and medium light levels. 5°C had the highest mean survival of all the temperatures, mean survival decreased with increasing temperature (Fig. 3.9). The wet treatments had higher mean survival than the damp treatments (Fig. 3.10).

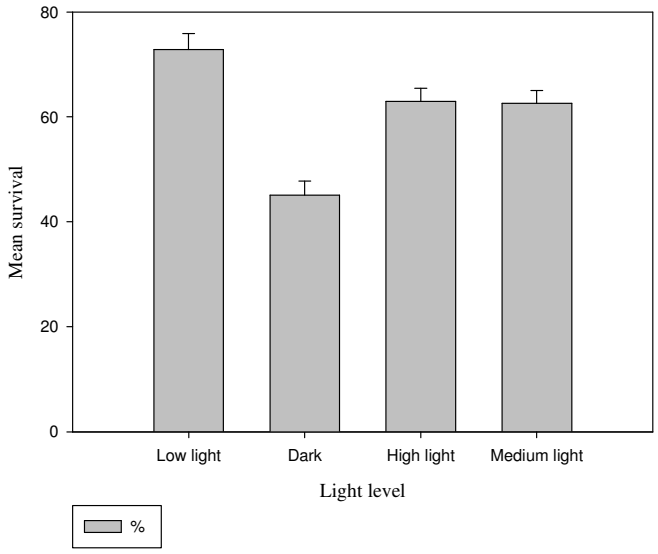

Fig 3.8. Mean survival time for *D. geminata* at different light levels. Low light had the highest survival rate over all temperatures and moisture levels, however this will be skewed as the low light was only present in two trials not four. High light and medium light have the same rates of survival.

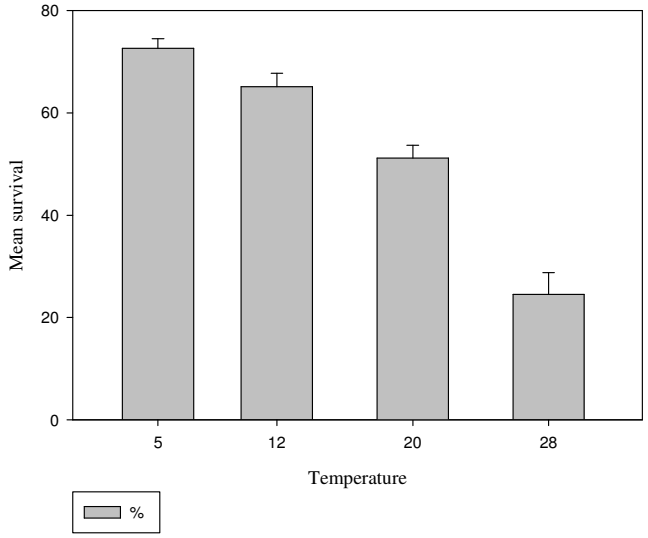

Fig. 3.9 Mean survival times for *D. geminata* at different temperatures. Mean **s**urvival increased with a decrease in temperature.

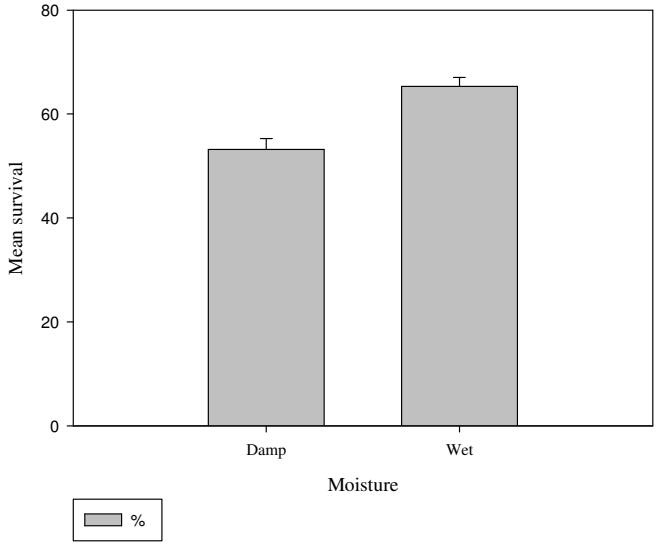

Fig. 3.10 Mean survival times for *D. geminata* at different moisture levels. The wet treatment had a higher mean survival.

3.4 Discussion

3.4.1 Temperature

Temperature was the strongest variable affecting the survivability of *D. geminata*. Samples lasted up to 60 hours at 28 °C, a decrease in temperature by eight degrees and *D. geminata* can survive up to 800 hours. Another decrease by eight degrees and all treatments can survive from 800 to 1500 hours. However, there is almost no difference in mean survivability of *D. geminata* at 5 and 12°C. These temperatures could be its optimum temperature, or the cells could have entered a resting state.

Rajadurai *et al.* (2005) found that phytoplankon cells could acclimatise to temperatures 10°C above their optimum growth temperature. However some species experienced a decline in growth rate. This would suggest that if *D. geminata* has an optimal temperature of 10°C, then above 20°C it would struggle to survive, which is consistent with the results. The 5 °C trial is within 10 °C below the optimum, therefore cells can survive.
Kilroy *et al.* (2006) tested *D. geminata* in freezing and hot (40°C) conditions, at both temperatures there was 100% mortality over short periods of time, which shows the limits to the survivability of *D. geminata*.

Kilroy *et al.* (2006) predicted that under wet conditions cells exposed to high, medium and low light treatments would survive up to eight months. This has direct implications for management of this species and other algae with similar characteristics. Border controls have to consider the longevity of microscopic cells when inspecting gear for importation into New Zealand.

Butterwick *et al.* (2005) imply that temperature is a factor influencing the quality and quantity of phytoplankton in cooler upland waters. *D. geminata* has been described as a cold water diatom, which is native to high altitudes in Britain. Possibly this diatom is restricted to these conditions in Britain due to other factors, such as water quality and substrate material. New Zealand may provide a greater range of habitats, the warmer temperature of the rivers could cause longer duration of bloom conditions. Another possibility is that *D. geminata* has adapted to various environments during its global dispersal, similar to the *Caulerpa taxifolia* invasion of the Mediterranean Sea (Meinesz 1999).

The cells in the medium and high light treatments at 12 and 5 °C began to grow and reproduce. Kilroy *et al.* (2006) suggest that *D. geminata* has never been grown in laboratory conditions. Yet in these experiments in more than one treatment, more than one replicate the cells grew, which was unpredicted from previous research. This aspect requires further research.

3.4.2 Light

Light in this experiment was the least important variable for the survivability of *D. geminata*. Cells in dark conditions were predicted to die quickly as they are unable to photosynthesise. This was certainly true for 20°C and 28°C at which cells were all dead within 200 h, however, at 12°C and 5°C cells survived up to 1500 h. This relationship has been found in marine diatoms, the colder the temperature the longer cells survive in the dark, unless the species is adapted to warmer water (Peters 1996). The diatoms in Peters experiment did not survive as long as *D. geminata*, but the pattern is similar.

The low light (blue shade) treatments were tested at 12 °C and 5 °C. The cells survived to the end of the sampling and had a high mean survival time, which indicates that *D. geminata* can survive to some extent in shaded conditions. *D. geminata* could possibly survive the low light conditions at 1-2 m under water in the middle of the river, increasing the possible coverage of the river bed. *D. geminata* could survive in shallow shady areas, which again would increase the area of the river that can be colonised.

Light reductions of up to 60-80% have been found not to affect the taxonomic structure of diatom communities (Peterson 1987). Severe light limitation of benthic algae is not evident until light intensity is reduced below 90% of ambient light. Survival at this reduced light treatment is normal for diatoms, however shading such as this has been found to cause moderate reductions in biomass (Peterson 1987 and see chapter 2).

The high and medium light treatments survived well at 40-80% live cells over 1500 hours at 12°C and 5°C. This length of survivability was unexpected. The original hypotheses were based on survival in earlier experiments and prior international knowledge.

3.4.3 Moisture

Samples survived better in wet than in damp conditions, especially at warmer temperatures when they survived twice as long as the damp samples. Once the clump had dried in the damp treatments cells did not recover with re-immersion in water. As in the present study, Mosisch (2001) found that diatoms survived better in wet than in damp conditions in Australian streams. He found diatom assemblages could not tolerate 15 days without water and re-immersion did not stimulate recovery. The majority of diatom species are not able to survive desiccation. However Peterson (1987) found that diatoms in direct stream flow had higher resistance to desiccation than diatoms sheltered from direct flow. He also found that diatoms in the direct flow produced more mucilage and therefore could tolerate desiccating conditions.

3.4.4 Management Implications

A possible reason for the variability within the replicates originates from the initial cutting of the *D. geminata* mats. The 10 x 10 x 5 mm samples were not identical. When they were cut off the mat attempts were made to ensure that each had about equal abundance of healthy cells. However, this was not entirely possible. The difference between samples was not predicted to be so variable. This variability is useful knowledge for design of policy and protocols, because an average time for survivability is not adequate information. When designing protocols the time of survival must be the longest possible so that no viable cells are dispersed into new environments.

3.5 Conclusion

D. geminata is able to survive long periods in marginal conditions. In cool to cold conditions, *D. geminata* can survive for months with a little water. Colder temperatures can increase survivability in the dark. It is possible that cells would remain viable on animal surfaces for considerable time. Cells in this experiment were able to survive for weeks in warm, damp conditions. Similar conditions could be found on bird feathers, and on fur or hooves of animals.

Human vectors such as kayaks could disperse large numbers of cells. If a kayak was not cleaned and then left in a cool damp place the cells could survive for months, as the 12 and 5ºC experiment illustrated. Fishers' clothing could also transport cells in damp conditions for a

number of months at temperatures of 12^{o}C or less. In light of this information potential human dispersers would need to take careful precautions to avoid further spread of this diatom. The longevity of its survival out of the river increases the risk of spread throughout New Zealand and the world.

Chapter 4: International and New Zealand policy

4.1 Introduction

Our mobile society is redistributing species across the globe at a pace that challenges ecosystems, threatens human health and strains economies (Vitousek *et al.* 1996, Vitousek *et al.* 1997). Biological invasions have become so widespread that they constitute a significant component of global environmental change (Vitousek *et al.* 1996, Mack *et al.* 2000). Invasion is a major threat to native biodiversity and equal to that posed by habitat loss and climate change (Levine and D'Antonio 2003, Hewitt, Willing, Bauckham, Cassidy, Cox, Jones, and Wotton 2004, Margolis *et al.* 2005). Invasive species can promote the extinction of genetically distinct populations, which is the least reversible of all human mediated global changes (Vitousek *et al.* 1996). In some regions of the world, as many as 80% of endangered species that are threatened or at risk have that status because of pressure from invasive species (Pimentel *et al.* 2005).

First, this chapter describes the problem of increased invasions due to international trade, and the international policies that impact upon trade regulations relevant to the spread of non-indigenous. Second, it outlines how New Zealand policy fits into global policy. It then goes on to outline national policies covering importation of new species and accidental introduction of new species, with specific examples in the marine sector. Risk management/strategies are then reviewed in detail.

4.2 Invasions and international trade

The geographic scope, rate of invasions and the number of species involved has grown enormously as a direct consequence of expanding transport and commerce (Bright 1999, Mack *et al.* 2000, Coutts and Taylor 2004, Work *et al.* 2005). These invasions are occurring at an unprecedented pace (Guo 2006), and invaders are collectively altering global ecosystem processes (Vitousek *et al.* 1996, Mack *et al.* 2000). It has even been considered that, "Bio-invasion has become a type of globalisation disease." (Bright 1999).

Elton (1958) noted this pattern, "We are artificially stepping up the rate of invasions, and feeling the manifold of consequences". The number of species that has entered new ranges through human agency has increased by orders of magnitude in the past 500 years and especially so in the last 200 years (Mack *et al.* 2000). These introductions by humans are both accidental and deliberate (Mack *et al.* 2000), and are continuing to be discovered (Work *et al.* 2005). Non-indigenous species can dominate landscapes in many parts of the world. The propagation of 'beneficial' species and the inadvertent spread of 'pest' species by humans are the two most powerful instruments of biological invasion (Didham, Tylianakis, Hutchison, Ewers, and Gemmel 2005).

The degree of risk for any country acquiring new species is linked to the volume of trade. There is a positive correlation between the number of invasive species and the volume and composition of imported goods (Perrings *et al.* 2005). Relating the number of new introductions to trade is complex, because the relationship is nonlinear. Container ships can transport whole suites of non-indigenous species. It is possible for some ships to introduce numerous species from many countries to a single port (Levine and D'Antonio 2003).

Arrival rates in maritime cargo in the USA have been found to be greatest in refrigerated containers carrying agricultural commodities (Work *et al.* 2005). A new species was found with every 50.5 inspections. Insects were intercepted on 26 different agricultural commodities, with peas having the highest interception rates. 68.9% of insect interceptions for the eight commodities shipped as air cargo were associated with cut flowers (Work *et al.* 2005).

However, in New Zealand the container review 2003 (MAF 2003a) found on average that in door inspections of 10,285 loaded containers 4.5% had some internal or external contamination. This ranged from 41% from Pacific Islands to 15% from Japan. The main external contaminant was soil, which was most common on the bottom of the container. Contamination rates were lower for empty containers. Interestingly, it was noted that: "Contamination rates for loaded containers with and without cleaning certificates did not vary significantly…" (MAF 2003a). Live organisms were rare with 0.7% on the exterior but up to 14.8% for the interior of containers. Many of the organisms found were already resident in New Zealand and therefore not regulated.

Despite the high number of species found in new sites, international regulations are not in place to ensure that exporters and importers are made responsible for their unintended cargo. The people responsible for introducing new species are seldom accountable for their actions (Perrings *et al.* 2005). A prime example of dispersal irresponsibility is the golden apple snail (*Pomacea canaliculata*) (Vitousek *et al.* 1996).

The golden apple snail was introduced into Taiwan to increase export income for small rice farms and to provide a supplemental source of protein. The snail proved to be distasteful and they consumed young rice plants. The snails grew rapidly and spread throughout irrigation canals. The export market of golden apple snails from Taiwan was closed due to health concerns. The entrepreneurs who originally imported the snail from South America simply continued to export it to most of the Far East and south-eastern Asia. They have not been made accountable for their actions (Vitousek *et al.* 1996, Vitousek *et al.* 1997).

In order to confront exporters who are the source of risk, with the costs of their actions, invasion externalities need to be internalised. Which means the cost of traded goods do not as yet reflect the cost of environmental damage. A risk-related tariff could reduce import volumes of risky species (Margolis *et al.* 2005, Perrings *et al.* 2005).

4.3 Global trading policy

The World Trade Organisation (WTO) regulates the international trading system. The rules that the WTO apply impact upon the environment, health and labour standards (Mullins 2000). The WTO prohibits policies discriminating against foreign goods, but such rules take no account of market failures, including the spread of non-indigenous species where the transport of the goods itself does the damage (Margolis *et al.* 2005). Additionally, measures have not been devised to prevent environmental protection policies from being overturned in trade disputes. These powerful international policies encourage invasion and the national and international mechanisms needed to control the spread of non-native species remain undeveloped (Bright 1999).

WTO decisions are narrowly focused and rarely take account of the broader goals of social welfare, development and security that trade is supposed to promote (Mullins 2000). Other

systems that are made for such purposes could be used for biosecurity purposes. The Container Security Initiative (CSI) is a USA programme designed to reduce terrorism against infrastructure. The system is designed so that many countries can have enhanced shipment security[4]. CSI consists of four core elements:

- Using intelligence and automated information to identify and target containers that pose a risk for terrorism and shared with trading partners on a bilateral basis.
- Pre-screening those containers that pose a risk at the port of departure before they arrive at U.S. ports.
- Using detection technology to quickly pre-screen containers that pose a risk.
- Using smarter, tamper-evident containers.

A similar system could be devised to check for travelling species if countries could see biosecurity issues as important as political security.

The International Plant Protection Convention (IPPC) of 1951 sets out regulations concerning quarantine against crop pests (Mack *et al.* 2000).

- "Contracting parties shall cooperate in the exchange of information on plant pests and their occurrence, outbreak or spread, especially if international action is needed".
- "Contracting parties should participate in so far as practicable, in any special campaigns, emergencies and provide information where necessary for pest risk analysis." (Perrings *et al.* 2005)

However, actions that are practicable for high income countries might not be so for those of low income (Perrings *et al.* 2005). The IPPC was revised in line with the SPS agreement in 1997 (Hayes 2003).

The Sanitary and Phytosanitary Measures (SPS agreement) was introduced to help combat invasive species. The initial policies were formed in 1994 at the end of the Uruguay round of multilateral negotiations. This agreement allows countries to enforce measures to prevent the spread of disease and pests (Hayes 2003). The SPS agreement was fashioned to balance the interests of nations in keeping out threats to plant, animal, and human health, including those

[4] http://en.wikipedia.org/wiki/Container_Security_Initiative 30/5/07

posed by introduced species, without impeding international movements of goods (Simberloff 2003).

The SPS agreement contains three primary disciplines:

1. National SPS measures must be based on risk assessment.
2. SPS measures are non-discriminatory between trading partners.
3. SPS measures must not be more trade restrictive than necessary (Mullins 2000, Thompson Campbell 2001).

All SPS measures must be based on scientific evidence, although provisional regulations can be adopted while seeking more scientific information (Thompson Campbell 2001, Margolis *et al.* 2005).

The SPS agreement is strongly influenced by the precautionary principle approach. This is reflected in the 1992 Rio Declaration on Environment and Development which states: "Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost effective measures to prevent environmental degradation" (Mullins 2000). Essentially this means that political responses to avoid environmental damage can be made without scientific certainty as certainty may come too late or not at all. This principle is sited in cases where there is the potential for serious or irreversible harm including pollution, threat of species extinction or the introduction of harmful products into the environment (Mullins 2000).

The SPS agreement also includes the concept of Appropriate Level of Protection (ALOP). Countries can decide the level of protection required for different products and traded goods. There is no limit to the level of protection a nation may seek including "zero risk" (Margolis *et al.* 2005). However, the country must consistently apply comparable levels of protection in comparable situations, and requires scientific justification for the level of protection (Thompson Campbell 2001). ALOP can also be termed acceptable or managed risk.

When species have been designated as a quarantine pest**,** the agreement requires phytosanitary agencies to ensure that there is minimal disruption to trade. However, if the WTO finds that there are insufficient scientific grounds for the SPS measures it will intervene and impose penalty fees (Mullins 2000). This occurred in 1989 when the European Union (EU) was penalised US$124 million per year for not importing beef, which had been fed growth

hormones, from the USA and Canada. The WTO also allowed the USA and Canada to impose 100% tariffs on selected European goods. The EU were able to sustain the consequences of maintaining its regulations, however, smaller countries would not be able to resist WTO intervention (Mullins 2000).

Inevitably there is a balance between the risk of pest and disease incursions and the benefit of trade. A country with a conservative quarantine policy minimises the potential for incursions but sacrifices trade benefits. The additional costs for such policies include maintaining larger border services and restricted access to products from other countries. Any decrease in protection leads to the potential rise of incursions and increase of the associated costs to industry and the environment (Wilson 2000).

This balance or trade off can also be applied to the tourism industry, the more international tourists who enter and tour a country the higher the risk of incursions. Locals then spread established pests throughout the country, but do not introduce new species. However restrictions on tourism may reduce revenue. Von Holle and Simberloff (2005) suggest that "any reductions in the rate at which propagules of non-indigenous species arrive at sites are likely to reduce the probability that invasions will succeed."

4.4 New Zealand Biosecurity Policy

4.4.1 Structure and function

Biosecurity has been defined in New Zealand as "protection from the risks posed by organisms to the economy, environment and people's health through exclusion, eradication and control" (Jay, Munir, and Bell 2003). In addition, biosecurity responsibilities include audit and enforcement of legislation, as well as providing sanitary and phytosanitary assurances to trading partners (Hewitt *et al.* 2004). There are five components to New Zealand's biosecurity system: border control, import health standards, post-entry quarantine, surveillance, and emergency disease and pest response. Pest response includes post-border pest management strategies (Hayden and Whyte 2003).

4.4.2 Border control

Most invasions begin with the arrival of a small number of individuals (Mack *et al.* 2000). Costs of excluding these initial populations are usually trivial compared to the cost and effort of control after populations have grown and established. Pre-border measures include agreements with other countries on disease reporting procedures and treatment of imported goods (Jay *et al.* 2003). Border control in New Zealand is coordinated by Biosecurity New Zealand which is an autonomous entity within the Ministry of Agriculture and Forestry (MAF) and New Zealand Customs Service which aims to restrict the entry of unwanted organisms to an acceptable level (Jay *et al.* 2003).

All persons (around 9000 per day) who land at international airports have their luggage x-rayed, and are subjected to the quarantine detector dog programme which supplements the x-ray inspections. All mail entering the country goes through Auckland mail centre. The mail is then x-rayed and checked by dog teams (Hayden and Whyte 2003).

Containers arriving at ports are inspected based on four components: exterior, interior, packing and packaging within container and cargo. No cargo will be removed until it has been approved by MAF. High risk containers are either thoroughly inspected by a MAF officer, or fumigated with methyl bromide or decontaminated by other approved method or accompanied by an international certificate that states that the container is free from contaminants. Inspection can be arranged before shipping as part of a bilateral agreement (MAF 2003b).

4.4.3 Import Health Standards

Import Health Standards (IHS) cover the importation of risk goods, "which are defined as any organism, organic material, or other thing or substance, that (by reason of its nature or origin) it is reasonable to suspect to constitute, contain, or otherwise pose risk that will result in:

a) exposure of organisms in New Zealand to damage, disease loss or harm; or
b) interference with the diagnosis, management or treatment, in New Zealand, of pests or unwanted organisms." (Hayden and Whyte 2003)

Any risk goods which enters New Zealand enters unconditionally, this means once introduced there are no restrictions on the spread of the organism. Therefore, before giving biosecurity clearance inspectors must ensure that the goods are not a biosecurity risk. There is no need to have an IHS for all risk goods and an IHS will only be issued when sufficient requirements can be imposed to provide for effective management (Hayden and Whyte 2003).

An import permit must be obtained to import species intentionally. Environmental Risk Management Authority (ERMA) issues specific import permits for legal importations, and operates under the Hazardous Substances and New Organisms Act 1996. ERMA's requirements are very stringent. Most permits issued by ERMA are for plants and animals in containment, such as zoos or research facilities (Hayden and Whyte 2003). Included under the new organisms part of the act is Genetically Modified Organisms (GMO). GMO's are permitted into the country under strict controls, the material is not allowed to escape until it has been consented.

4.4.4 Post-entry quarantine

Post-entry quarantine treats plants and animals according to the risk factor associated with their country of origin. In some cases just an import certificate is adequate. In other cases goods are restricted or banned depending on the item and the country of origin (Hayden and Whyte 2003).

4.4.5 Surveillance programmes

Surveillance programmes are used to monitor the health status of New Zealand's flora and fauna including export crops. The programmes credibly certify New Zealand's true pest status. The programmes reduce sanitary requirements for export, enable prompt notification of exotic diseases, support pest management strategies and assist with public health policies (Hayden and Whyte 2003). Pine pitch canker (*Fusarium circinatum*) is a topical example, as the forestry industry grows in New Zealand the risk of contracting disease increases. This disease is fatal to trees from nursery stock to established populations. If the disease arrived in New Zealand the consequences would be damaging to the economy, as treatment would be required to export products, which can be costly[5].

4.4.6 Pest and Disease management strategies

Pest and Disease Management Strategies (PDMS) are designed to manage or eradicate pests which are present in New Zealand. All PDMS require approval from the Minister of MAF to ensure that they are feasible, safe and affordable (Hayden and Whyte 2003). Included in PDMS are regional councils and section 100.

[5] http://www.biosecurity.govt.nz/bio-strategy/biostrategy.htm#surveillance

PDMS are underpinned by the Biosecurity Act 1993. Regional councils must undertake a PDMS in order to start pest control or use section 100 provisions. Section 100 is used for small scale management of unwanted organisms if,

a) "an unwanted organism present in the region could cause serious adverse and unintended effects unless early action to control it is taken; and
b) The organism can be eradicated or controlled effectively by small-scale measures within three years of commencing measures to control the organism, because:

- Distribution of the organism is limited; and
- Technical means to control the organism are available"[6]

Otherwise the incursion has to be reported to Biosecurity New Zealand and they apply to Cabinet for funding to combat the new species. This can take years or not at all depending on the costs and priorities of the current Cabinet. For example, Dutch elm disease exists in Auckland. It currently has a PDMS, however for eradication to be successful the existing programme needs a significant increase in resources. Biosecurity New Zealand submitted a new bid seeking additional funds, the request was not successful[7].

New Zealand has been successful in eradicating some species, an example is the tussock moth (*Orgyia thyellina*). Tussock moths were discovered in Auckland's eastern suburbs on April 17th 1996 (Hosking *et al.* 2003). The Auckland area was sprayed by a DC6 aircraft and a BK 117 helicopter with an organic insecticide (*Bacillis thuringiensis* var. *kurstaki* toxin), which eradicated the moth from New Zealand at a cost of $5 million. Post-eradication monitoring was established to ensure no more moths were found. Caged females were set in sticky traps to catch males. If males were found in the traps then the local area was sprayed again. No males were caught for a period of two years, allowing New Zealand to say that it is free of tussock moths (Clearwater 2002, Simberloff 2003).

The five aspects (Border control, IHS, post-entry, surveillance and PDMS) of Biosecurity in New Zealand mean that the number of new species establishing in New Zealand is reduced. Biosecurity has now become a high priority for politicians and the public, resulting in funds being made available for fighting new incursions. However, there are still problems with

[6] http://www.arc.govt.nz/arc/index.cfm?F6D4A46B-BCD4-1A24-915A-BAE95853B362
[7] http://www.biosecurity.govt.nz/files/publications/biosecurity-magazine/issue-61/biosecurity-61.pdf

marine and freshwater areas as little is known about established populations. This hinders responses to new invasions.

4.5 Marine Policy in New Zealand

The Ministry of Fisheries (MFish) has had a marine biosecurity team since 1998. The team has developed a risk management framework (RMF) to evaluate the critical system risks to biosecurity. It focuses on: healthy environments, strong communities, vibrant commerce and high quality recreation (Hewitt *et al.* 2004). An example of risk management in New Zealand is biofouling. Biofouling on international vessels is an important pathway for the inadvertent movement of species around the globe and into New Zealand (Coutts and Taylor 2004).

4.5.1 Pre-border Management

Currently in pre-border management there is an Import Health Standard (IHS) for international ballast water which requires "ballast water to be exchanged in mid-ocean and no discharge of un-exchanged waters from any country unless exempted. However, no discharges of un-exchanged water sourced from Tasmania and Port Phillip Bay, Victoria, Australia are permitted." (Hewitt *et al.* 2004). This unilateral agreement was one of the first in the world. Pre-border management of hull fouling is education to maintain clean hulls, there is no specific policy surrounding this vector.

4.5.2 Border

The marine biosecurity team has limited means to manage hull fouling of international shipping. Currently hull fouling is not inspected at the border but officers collect information that is used to determine risk. Shipping can disperse species through a variety of mechanisms including, ballast and bilge water discharges, biofouling or hull fouling, sea chests, sea sieves, anchors, chain lockers and piping (Coutts and Taylor 2004).

4.5.3 Post-border

Post-border control in marine areas is difficult as there is little knowledge on what naturally occurring marine species there are in New Zealand. Therefore baseline surveys of what already exists was undertaken and repeated after three years to evaluate the current rate of

invasion. Constant surveillance in high risk areas has been established for early detection of incursions. As a result, the rate of detections and reporting has increased significantly since 2000 (Hewitt *et al.* 2004). The capability of the marine biosecurity team to detect species at the border and post-border relies heavily on taxonomic information, which is limited in many marine groups.

Many of the ships moving to and from Australia were the worse for biofouling compared to other international ships arriving in New Zealand. These trans-Tasman vessels had the highest proportion of anti-fouling paint older than 36 months (Coutts and Taylor 2004). They conclude that although these vessels have introduced a number of species, to date the effects of their introduction appears to be relatively benign.

Freshwater incursions are also problematic as Duggan, Green, and Burger (2006) found. Freshwater zooplankton are establishing in New Zealand from the northern hemisphere and Australia through aquaculture and the transport of tropical water plants. The main vector for the zooplankton seems to be hitchhiking in the water on plants. Non-indigenous zooplankton may markedly affect lake ecosystems because of their place at the base of the aquatic food web, and yet the effects of such invasions are poorly documented (Duggan *et al.* 2006).

4.6 Risk assessment /strategies

Many plant taxa are still being imported purposely into New Zealand and Australia, which may have the potential to become agricultural or environmental weeds (Pheloung, Williams, and Halloy 1999). This risk has to be assessed before allowing their entry, especially as most plant invaders that threaten native ecosystems have been introduced intentionally for horticulture, forestry and other human uses (Daehler, Denslow, Ansari and Kuo 2004). Pheloung *et al.* (1999) describe a weed risk assessment as a system that uses information on a taxon's current weed status in other parts of the world, climate and environmental preferences, and biological attributes. Daehler *et al.* (2004) found the noxious weed lists contain predominantly agricultural pests and few environmental pests, which means that the screening systems allows the entry of many plants that threaten native ecosystems.

There are a number of risk assessment systems and a variety of opinions on the subject (Levin 1989, Parker, Simberloff, Lonsdale, Goodell, Wonham, Kareiva, Williamson, Von Holle, Moyle, Byers, and Goldwasser 1999, Pheloung *et al.* 1999, Daehler *et al.* 2004). Levin (1989) suggests that the first step to an assessment of an introduced species is the identification of the primary modes of movement into new areas, and the scale of these movements. Risk assessment systems should satisfy the following requirements, they should be calibrated and validated against a large number and variety of established organisms which represent the full spectrum of taxa likely to be encountered as imports (Pheloung *et al.* 1999).

Parker *et al.* (1999) suggest there are three stages to a risk assessment, identifying the hazards, quantifying them and then allowing for values and the perception of risk. Daehler *et al.* (2004) further suggest that an ideal system should have four key properties:

1. Components should have a scientific basis;
2. System should be transparent - that is, why a species is defined as a pest;
3. The assessment process should minimise the use of subjective opinions;
4. Assessment must be able to be replicated.

Additional information can be useful for screening programmes and prediction of invasive species, which combined could be formidable in the battle against invasive species and their control. For example, information on abiotic limiting factors in native ranges can help us predict areas and habitats into which invasive species might expand (Guo 2006). Information on low-impact invasive species will help identify characteristics that set high-impact non-indigenous species apart, giving greater understanding of the relative harm a new introduction can cause (Byers, Reichard, Randall, Parker, Smith, Lonsdale, Atkinson, Seastedt, Williamson, Chornesky and Hayes 2002).

Guo (2006) noted that information from native populations and habitats are critical for investigating the causes of species invasiveness and for developing control measures, this comparative approach is a potentially powerful tool. However, the best predictor of whether a non-indigenous species will have negative effects is if the species had been a pest in other places (Simberloff 2003). He also suggests when the biology of most species is known, risk assessment could be useful in pinpointing proposed introductions needing close attention. The geographic range that a species naturally inhabits is an important indicator of invasiveness, if a

species can tolerate a wide range of environmental conditions they are more likely to succeed in a new environment (Goodwin, McAllister, Fahrig 1999).

Pheloung *et al.* (1999) recommended that ideally the system should be capable of identifying which land use system the taxon is likely to invade, to assist in an economic evaluation of its potential impacts. Bax, Hayes, Marshall, Perry and Thresher (2002) agree that risk assessments are rigorous and systematic, however they are time consuming. As Andersen *et al.* (2004) explain, ecological impact assessment is further hindered by the lack of a common currency for measuring and expressing changes, by uncertainty or disagreement about what constitutes an adverse ecological impact.

As useful as screening programmes and risk assessment is, a wide range and large number of species arrive into new locations as accidental introductions. For this reason Marvier, Kareiva, and Neubert (2004) suggest that it would be more useful to lump exotic species in a common pool and think of them as a form of biological pollution, which should be minimised at the ecosystem level. Goodwin *et al.* (1999) further state that prediction based on species-by-species approach is unlikely to help stem the flow of species moving around the globe. Marvier *et al.* (2004) agrees with this sentiment but qualifies it by saying that the information gained by the species-by-species approach is valuable in building up generalities about invasive species. Ding, Reardon, Wu, Zheng, and Fu (2006) have shown that it is possible for countries to work with each other to reduce the risks of invasion. Additionally these countries have the benefits of exchanging information on common invasive species, and collaboration on biological control.

But in reality even with all the information known on an individual invasive species, the issue weighted by public concern, resource managers are still faced with limited budgets and numerous priorities (Parker *et al.* 1999). Money and resources are always limiting so managers must decide which populations of invasive species to control immediately, which populations to control when time and money permits and which populations are not important at present (Byers *et al.* 2002). However, as Andersen *et al.* (2004) explains the authorised risk managers are commonly government officials, therefore most risk based decisions concerning invasive species are public policy choices.

Policy decisions for invasive species should be based on clear scientific reasoning, but social, economic and political concerns should also be addressed, even if it complicates things (Byers *et al.* 2002). Maguire (2004) outlines a number of stake-holders involved in the decisions for the control of feral pigs in Hawaii, these include; land managers of national parks, managers of state natural area reserves and private nature reserves, animal damage control personnel, game managers, native Hawaiians interested in medicinal plants, native Hawaiian pig hunters, and animal rights activists from Hawaii and elsewhere. All their needs have to meet with the best management of this invasive species control

Parker *et al.* (1999) imply the importance of public awareness and perceptions of risk, when dealing with invasive species and their control or eradication management. Some invasive species have a positive public perception and others have negative, even when both have effects on the environment (Maguire 2004). Maguire (2004) contrast lady beetles with kudzu, public like the former invaders and not the latter. Currently the public in New Zealand has a negative perception of *D. geminata* as it is not aesthetically pleasing and detrimental to a number of stakeholders. This negative perception reduces conflicting opinions when making important biological, economic and political decisions.

Accidental introductions are just as difficult to control as purposeful introductions. Risk assessments, programmes designed to work with surveillance and funding would reduce the number of accidental introductions. Accidental introductions can be more difficult to control as the pathways into the country are not always clear and policy is not always available to stop the incursions. *D. geminata* fits into the accidental category. The introduction of *D. geminata* to the South Island of New Zealand has had consequences for the environment, the economy and recreational users of the areas. Bax *et al.* (2002) thwarted an accidental invasion by a relative of the zebra mussel.

The Caribbean black stripped mussel (*Mytilopsis sallei*) was discovered in Cullen Bay Marina, Darwin, Australia on March 27th 1999. April 2nd 1999 extensive diver surveys were undertaken to determine the densities of the mussel, they found up to 23 650 individuals/m^2 which were not present six months earlier when the last baseline survey was conducted. Chlorine (160 000L of liquid bleach) and copper sulphate (6000 tonnes) treatment began on April 4th and continued to April 8th (Bax *et al.* 2002, Simberloff 2003). This treatment was conducted in closed marinas, which have double lock gates, boats in the marina at that time

were also treated externally and internally. By the 9th of April 1999 the mussel had been eradicated from three bays in the Darwin area (Bax *et al.* 2002).

The speed that this invasion was dealt with has ensured that the mussel has not become another invasion statistic. A risk assessment was unable to be conducted in such short time but hazard analysis was conducted and was sufficient enough to know the hazardous situation this mussel could pose. However bleaching large areas is not always a viable option. As Simberloff (2003) so aptly said, there will be some failures when people act quickly without much research, sometimes this research might have predicted the failure or led to a different approach that would have succeeded. However quick and dirty responses, mechanical or chemical or both, often solves the problem at the outset by eliminating the invaders (Simberloff 2003).

4.7 Conclusions

There are considerable problems with invasive species and international trade. Policies aiming to reduce international invasions that impact upon trade regulations are becoming more evident as the consequences of invasion are more obvious and costly. The policies that New Zealand has been able to implement in the last decade have reduced the number and variety of incursions. Risk assessment is a useful tool in reducing the flow of invasive species purposely introduced into new environments. Risk assessments have to account for public concerns and political implications of any eradication and control of invasive species.

Chapter 5: Discussion and Management Implications

5.1 Introduction

D. geminata has entered New Zealand through international pathways from the Northern Hemisphere, through one or many vectors, and established in the South Island. The invasion has followed the steps and stages model (Section 1.4). This diatom overcame many scale dependant hurdles to establish and flourish in this new environment. It is unclear if *D. geminata* had a lag phase as the time of establishment is unknown.

The dispersal of *D. geminata* can be predicted using habitat characteristics. The South Island has more area that is suitable for *D. geminata* which is the reason for its bloom conditions in sites where establishes. The field experiment (Chapter 2) confirms this, sites with good substrate, temperature and velocity had the highest biomass. The test of survivability (Chapter 3) showed that *D. geminata* can survive and establish in a wide range of environmental conditions. For these reasons the North Island has some risk of establishment.

Policy has been formed to reduce the risk of continuing dispersal from international and local sources. This had limited success as the diatom continues to spread. However, *D. geminata* has affected policies at international, national and regional levels. *D. geminata* has also increased the public awareness about invasive species and has highlighted aquatic issues which has had little focus or investment. This study has shown the consequences of an invasive aquatic species and the policy surrounding its invasion and spread in New Zealand.

5.2 *D. geminata* and invasion theory

D. geminata has conformed to the steps and stages model suggested by Heger and Trepl (2003) (Table 5.1). *D. geminata* had to overcome scale dependant hurdles to get to the current status of a pest, which is still expanding into new areas. Some of the steps and stages have not been observed as the time of initial establishment is unknown.

Table 5.1 The steps and stages model of Heger and Trepl (2003)

Model steps and stages	**Steps and stages of the invasion of *D. geminata***
Step one, immigration	*D. geminata* arrived before October 2004, possibly as early as 1997. Probably on fishing clothes or equipment from the Northern hemisphere.
Stage one, presence in the new area	*D. geminata* was found established in the Waiau River in October 2004. This stage would have occurred some time before.
Step two, independent growth and reproduction	When *D. geminata* was found it was growing vigorously
Stage two spontaneous establishment	Not observed
Step three, growth to minimum viable population	Data is not available on the minimal viable population. However, small clumps used in the laboratory experiments had enough individuals to increase the population.
Stage three, permanent establishment	*D. geminata* was permanently established when discovered by Fish and Game in 2004.
Step four, colonisation of new localities	*D. geminata* has established in a number of sites around the South Island, and is continuing to spread.
Stage four, spreading in the new area is complete	This has not been completed, *D. geminata* is still dispersing.

A lag phase (between stage three and step four) may have occurred but as there is no formal information available on the date of establishment, a lag phase was not observed. However, there is some anecdotal evidence that *D. geminata* was noticed in the Waiau River by some Canadian fishermen in 1997 (F. Sullivan pers. comm.). If this was true then *D. geminata* may have had a lag phase of a few years. During this lag time only the fittest individuals survived in the new environment.

5.3 Vector analysis

The pathways for *D. geminata* into New Zealand are from North America and Europe. The use of molecular genetic markers is needed to show how many times *D. geminata* has been introduced. The variation in genes at specific loci should show how many different individuals have established in New Zealand. This information should show how important the international pathways are for dispersal into New Zealand, compared to local dispersal of established populations.

Vectors that disperse *D. geminata* around the country are similar to vectors that disperse internationally. Fishing equipment is the most likely candidate to disperse internationally as some clothing can stay damp for weeks to months if not cleaned and dried properly, such as

neoprene waders and felt soled boots. Cells can survive with little moisture and light for at least two months at cool temperatures (Chapter 3). When clothing is used again in a different country, different catchment or within a catchment the cells that have been attached to the clothing disperse into the new system. However *D. geminata* has a very variable survival time which makes it difficult to predict ease of dispersal by this vector. Once the cells have established in the new system they grow, spread and disperse by one of many vectors (Table 5.2).

Table 5.2. Six vectors that *D. geminata* could be dispersed internationally and nationally. There are three different ways that *D. geminata* can be transported: as abundant cells in a body of water, as small clumps and as a few individual attached to a surface. The vectors can then disperse at a range of scales, within the catchment, between catchments and internationally.

Vectors		Modes		
	Water (abundant cells)	Small clumps	Few individual cells	Area of dispersal
River clothing	Low	Medium	High	Catchment, National, International
River footwear	Low	Medium	High	Catchment, National, International
Fishing lines	Low	Medium	Medium	Catchment, National, International
Kayaks	High	High	Low	Catchment, National
Cars	Medium	High	High	Catchment, National
Animals	Low	Low	Medium	Catchment

The North Island has a reduced risk of establishment, as there are fewer high-quality habitats for *D. geminata* (Fig. 5.1). Compared with the South Island, the North Island has warmer temperatures and fewer braided rivers with large cobbles. Substrate size and stability is important for growth and bloom conditions (Chapter 2). These factors should decrease the risk of establishment. If dispersal and establishment does occur in the North Island, *D. geminata* is less likely to attain large biomass as there are less high-quality habitat available.

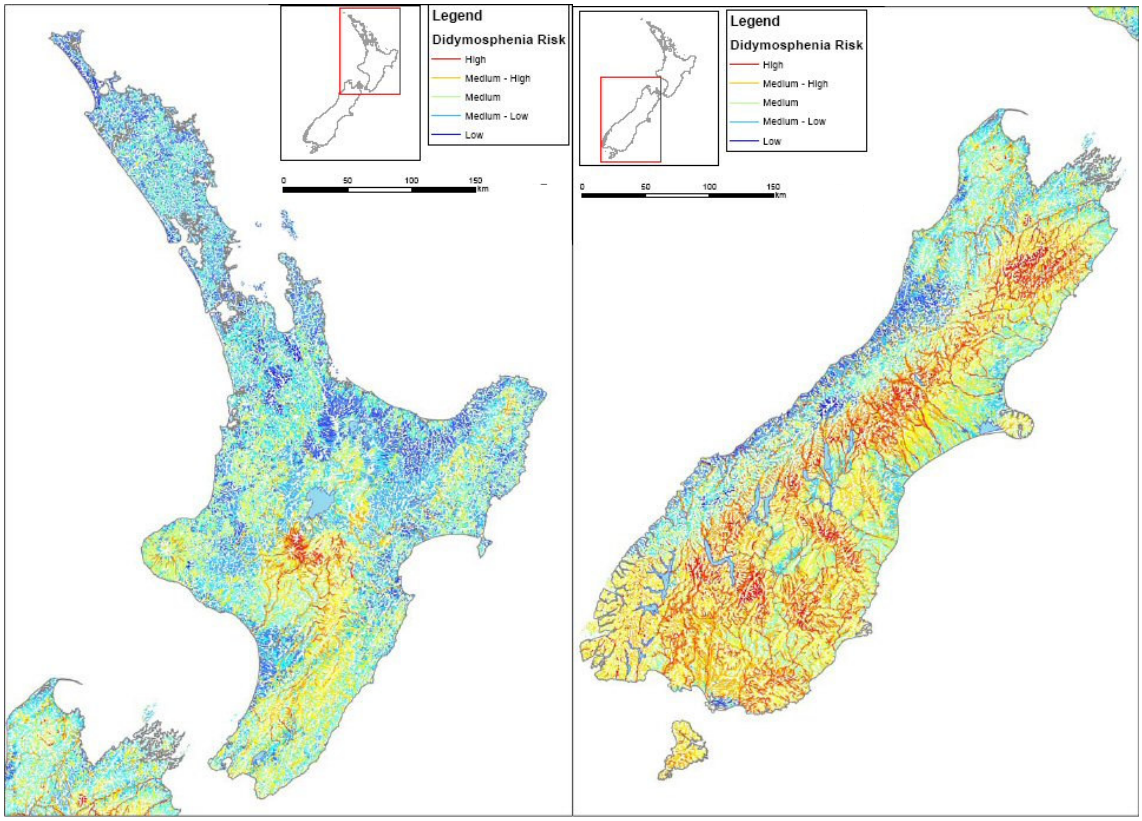

Fig.5.1 Map of the North and South Island coloured according to the risk of *D. geminata* dispersing and infecting new areas. Sourced from Biosecurity New Zealand[8].

The east coast of the South Island has higher risk of *D. geminata* dispersing and establishing (Fig 5.1). This is due to the cooler water temperature and the type of bed rock substrate, as the South Island is dominated by stony braided rivers. High-quality habitat in the South Island can produce blooms of up to 2.51mg mm^{-2} over six weeks during summer (Chapter 2). Site two had smaller stones and *D. geminata* was unable to establish easily as the stones were constantly moving with the flow of the river. This was especially evident when the flow was high and fast.

D. geminata is established in 52 rivers and five lakes covering nine catchments[9]. The pattern of spread of *D. geminata* throughout the South Island over the last three years shows that dispersal has not been random (Fig 5.2). Since starting at the Waiau River (seen as the blue star Fig 5.2), *D. geminata* has spread through the original catchments and downstream of the initial establishment. This suggests movement of cells by the river downstream and possible

[8] http://www.biosecurity.govt.nz/files/pests-diseases/plants/didymo/images/didymo-risk-map-south-island.jpg 30/05/07
[9] http://www.biosecurity.govt.nz/pests-diseases/plants/didymo/didymo-stakeholder-update-may-07.htm 11/06/07

animal vectors. New catchments in which *D. geminata* has established are common fishing spots. This is especially evident in northern catchments. Which are separated from other sites and separate from each other. This suggests that humans are dispersing the diatom over these long distances.

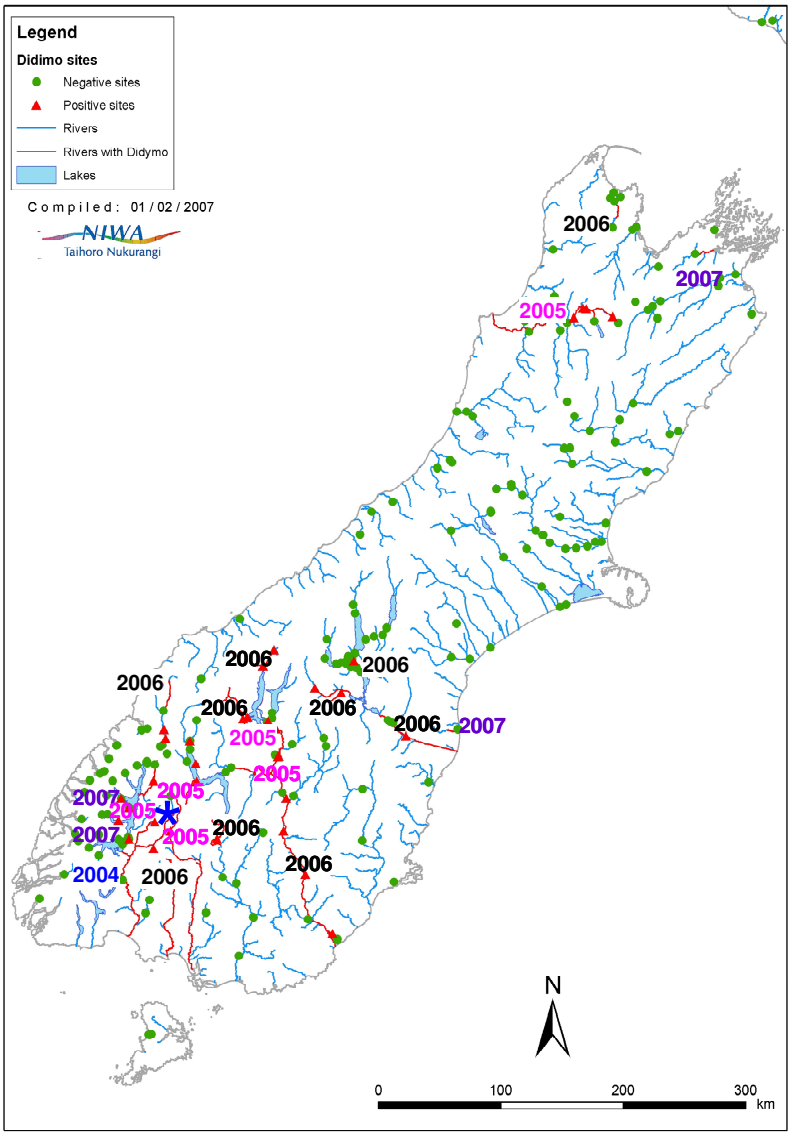

Fig.5.2 Map of the rivers and sites that are infected with *D. geminata*. This map shows the year in which each of the sites was infected. It is possible to see the pattern of spread through the years. The blue star indicates the point of initial establishment. (C. Kilroy unpublished data.)

5.4 Stakeholders

The stakeholders involved in the management and control of *D. geminata* are: environmental managers, fishers, farmers, Maori, the public, recreationists, Government (Cost of control and eradication) and tourists. Each stakeholder has an interest and opinion on control, eradication and management, which could be the opposite to that of other stakeholders. *D. geminata* is seen in a negative light by all stakeholders, which makes eradication and control generally easier.

Tourists and local fishers find *D. geminata* unsightly, they no longer want to fish in infected rivers (F. Sullivan pers. comm.). Infestations change the ecology of rivers and irrigation costs increase for farmers. *D. geminata* interferes with river sports and the degradation of the river greatly concerns Maori. Public perception does not object to the eradication of *D. geminata*, unlike the Kaimanawa horse herd, which has to be managed in the most delicate ways (Rodgers 1991). However, some of the options that are available to manage and control this invasion may not be viewed positively by some stakeholders.

Biosecurity New Zealand updates stakeholders, particularly the general public, with new information and cleaning updates via their web site.[10] Biosecurity New Zealand has implemented regional groups that will help them with prevention, detection and management of *D. geminata*. This move by Biosecurity New Zealand is to get the "Check, Clean, Dry" message through to as many people as possible.[11]

5.5 Exacerbators

In the South Island there is a strong interest in trout (Oncorhynchus mykiss, Salmo trutta) fishing. There are many tour operators who will take anyone throughout Tasman District, Central Otago, Central Lakes District, and Southland for trout fishing. The prime environments for this overlap with *D. geminata* habitat, hence the infection of rivers at fishing sites. Kayakers are also proving problematic. Kayaking tours go through similar territory as fishers. Water is taken on board when paddling and this is then transferred at entry and exit sites. If a

[10] http://www.biosecurity.govt.nz/pests-diseases/plants/didymo/didymo-stakeholder-update-may-07.htm 11/06/07
[11] http://www.biosecurity.govt.nz/pests-diseases/plants/didymo/didymo-stakeholder-update-may-07.htm 11/06/07

boat enters a water body from an infected site and exits from a non-infected site the exit point has a high risk of *D. geminata* establishment. Kayakers are also able to disperse *D. geminata* from one catchment to another. If the boats are not cleaned or dried properly there is high risk of dispersal into new areas when the boat is next put into the water.

Biosecurity New Zealand has the authority to issue fines for spreading *D. geminata*, however there is difficulty in proving guilt. This method of deterrent has not been successful as it is still spreading (Fig 5.2). Therefore a permit or tariff should be put into place. A permit would include education at the point of purchase including notification of fines for dispersal. Also, information on equipment decontamination would be provided. Tour operators would have to purchase a special permit for taking groups of people into the area and a course on decontamination would be essential. However, 100% compliance is an unrealistic goal as there is always someone who does not care, or individuals who cause damage purposefully. In recent survey by Fish and Game Nelson they found that of those surveyed 62% had heard of *D. geminata*, 50% had heard of "Check, Clean, Dry". However only 60% of people who knew about "Check, Clean, Dry" used the information and took action (N. Deans Pers. Comm.). This survey shows that even when people know about the problem and what to do about it they still choose not to 40% of the time. This percentage is probably the group that disperse *D. geminata* most often.

A tariff could be used in high risk areas. This would mean that in order to do high risk activities a tariff would have to be paid on entry to non-infected rivers and catchments. At the entry point where the tariff is paid, a full check of gear could be undertaken. The tariff option can be costly, but the income derived from the tariff should cover the cost of necessary staff. However, this approach does not account for animal vectors.

Another approach would be to invoke a sense of pride and responsibility for the environment, with specific recreational areas targeted. The public then would endeavour not to spread invasive species or at least think about consequences of their actions.

5.6 Quarantine approaches

In Australia, water articles and equipment must be declared on entry to the country and must be decontaminated. The Australian Quarantine and Inspection Service (AQIS) give a number of options that importers can choose depending on the article. Decontamination options vary from dishwashing liquid and hot water to gamma irradiation at 25 kGray (T9651)[12].

In New Zealand, all recreational equipment must be declared on entry to the country. The import health standard for equipment associated with animals or water (December 2005) states, "Equipment associated with water may be given clearance when the equipment is completely dry and has been declared clean and any equipment that has been used in the last 30 days has been cleaned by an approved method." Approved cleaning methods include; soaking in bleach or sodium percarbonate or sodium chloride, all for at least one minute. However, sodium chloride can be used only for decontamination of freshwater items, marine items must use one of the other methods. Felt soled shoes must be treated at the port of arrival or directed to an approved treatment supplier. Dive suits may be cleaned using a wet suit cleaner[13].

5.7 Risk management

Balancing the risk of environmental degradation by invasive species and income from trade and tourism is challenging. In New Zealand and Australia the AS/NZS 4360 is used as a model for risk management. Table 5.3 shows an overview of the risk management process for invasive species such as *D. geminata*.

[12] http://www.aqis.gov.au/icon32/asp/ex_casecontent.asp?intNodeId=8634153&intCommodityId=22867&Types=none&WhichQuery=Go+to+full+text&intSearch=1&LogSessionID=0 30/05/07

[13] http://www.biosecurity.govt.nz/imports/animals/standards/anieqpic.all.htm 30/05/07

Table 5.3 A risk management overview process for invasive species such as *D. geminata*.

AS/NZS 4360:2004 Risk management process overview	**Management of invasive species in New Zealand**
Establish the context, criteria against which the risk will be evaluated, and the structure of analysis defined.	Keeping New Zealand's environment from further invasions by accidental and/or purposeful introductions is of the utmost importance.
Identify risks, where, when, why and how events could prevent degrade, delay or enhance the achievement of the objectives.	Tourists arriving with organisms on their clothing and equipment are a significant risk and these events can happen at any time. These events could impact upon one of many environments throughout New Zealand. Tighter restrictions on persons travelling into New Zealand would reduce environmental degradation by invasive species. There is a trade off between short term income from tourism and possible serious long term environmental degradation.
Analyse risks, identify and evaluate existing controls. Determine consequences a likelihood and hence the level of risk.	Consequences of invasion by accidental species can be dire, as *D. geminata* illustrates. The risk of species getting through the current regulations is much smaller compared to pre-*D. geminata.* However these new controls still have risk associated with them.
Evaluate risks, compare estimated levels of risk against established criteria, consider pros and cons.	Increased restrictions on trade and tourism will benefit the environment, but the restrictions will have a negative impact on the economy. The opposite is also true, with increased trade the economy will benefit, while the environment degrades due to increased invasive species. The increase in invasive species could also have a negative impact on the farming industries.
Treat risks, develop and implement strategies which enhance benefits and reduce costs.	Importation of all equipment should be banned unless items are new or have been sufficiently cleaned by quarantine staff. This will increase benefits to the environment and protect against losses due to invasive species. However it may reduce tourism, as recreationists are unable to bring their sports equipment with them.
Monitor and review, monitor the effectiveness at each step in the management in the risk management process.	The criteria for species to purposely enter New Zealand are constantly updated. The restrictions on importations of equipment and clothing should also be under constant review. The restrictions should be based on past incursions and excluding current lists of invasive species.

5.7.1 Management options to prevent invasive species entering New Zealand by the vectors which may have introduced *D. geminata*

Option one

No recreational gear is allowed into New Zealand, without quarantine. This would include all fishing clothing and equipment, tents, hiking equipment, used sleeping bags and kayaks. Any equipment that is brought into the country would have to be decontaminated or destroyed at the importer's expense. People wanting recreational gear would either wait and pay for

decontamination (which could be lengthy and costly) or buy/ hire gear in New Zealand. This would increase revenue for the tourism industry via gear hire and sales. Large tourist operators could include gear hire in the price of their trips. This option has a low to zero risk of new organisms arriving and establishing in New Zealand through these vectors. This strict option may reduce tourism as some people may be deterred by the regulations. However, tourism that benefits the environment would be a useful tool in the conservation of New Zealand's unique ecosystems.

Option two

Some recreational gear is allowed into New Zealand. High risk equipment would be banned such as fishing equipment and clothing, but sleeping bags and hiking equipment excluding boots would be allowed. Any banned equipment would be either quarantined or destroyed. As part of the risk assessment for high risk equipment an assessment should be made on country of origin. This could make it easier to import equipment from some areas, with only a certificate necessary. Although people with multiple stops around the world will need a log of where and when their equipment was used and any cleaning that has occurred. This is a low to medium risk, as the risks will be assessed and hopefully the gaps will be near closed. The reduction in tourism should be less than in option one as it is easier to get their personal gear in New Zealand.

Option three

The *status quo,* allow most recreational gear into New Zealand. Customs officials check any gear that has been declared, and clean it if necessary. Risk associated with this option is medium, as many organisms can get through gaps. Tourists would find this the best option as nothing has to change and they are not required to do anything.

5.8 Policy implications and options

5.8.1 International

Australia has changed its importation rules as it desires to continue to exclude *D. geminata*. The tight controls on water articles and equipment from countries with *D. geminata*[14] are to reduce the risk of *D. geminata* dispersing into Australia.

5.8.2 National

D. geminata is a national problem which has been coordinated by Biosecurity New Zealand. During the last few years greater emphasis has been placed on invasive species. The policy surrounding importation of sporting equipment was amended one year after *D. geminata* was reported by Fish and Game. To reduce the risk of further incidents of its establishment and those of other micro-organisms. Policies for invasive species have to be flexible to cope with incursions of this magnitude.

Aquatic invaders such as *D. geminata* have increased public awareness of invaders. This is useful for Biosecurity New Zealand. They use the public as a surveillance tool to look for new invaders and to provide information on species spread. They have set up web pages and hotline numbers to report information regarding invasive species.

5.8.3 Regional

Freshwater systems are just as vulnerable to invaders as terrestrial environments. *D. geminata* has highlighted aquatic issues in regional areas. Currently there is little focus and investment in freshwater issues. For example, Environment Canterbury (ECan) has two water weeds in their regional pest management strategy 2005-2015 (Environment Canterbury 2005), compared with 11 animals, 25 plants and one insect, all of which are terrestrial organisms. This suggests that either there are only two problematic aquatic species in the region or there is a lack of information regarding aquatic species. ECan did assist Biosecurity New Zealand with management of *D. geminata* in the Waitaki River over the 06/07 summer, by increasing the awareness of *D. geminata* by placing signs and cleaning stations in prominent infected areas (Ecan 2007).

There is no general freshwater management plan, aquatic issues are dealt with on a species by species basis. Canterbury's water bodies are monitored according to the individual threats by

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14 http://www.aqis.gov.au/icon32/asp/ex_casecontent.asp?intNodeId=8634153&intCommodityId=22867&Types=none&WhichQuery=Go+to+full+text&intSearch=1&LogSessionID=0 30/05/07

invasive species. The water bodies themselves do not have management plans, except when registered unwanted organisms are present.

5.9 Conclusions

Invasive species are becoming more and more important on the international stage and they have highlighted the importance of biosecurity. Invasive species have social, economic and ecological implications. Species such as the Zebra mussel are causing ecological and economic disaster in many regions of the world, to the point where marinas are being bleached when related species establish.

D. geminata is becoming renowned for its ecological impacts. Gaining an understanding of the biology of *D. geminata* assists with management of the invasion. *D. geminata* is not the only invasive aquatic organism in New Zealand but it has emphasized aquatic issues. It has highlighted to the public and policy makers the consequences of invasive species in these fragile ecosystems.

Currently in New Zealand the increased restrictions on importation of equipment into the country are likely to be reducing the number of invasive species gaining entry through that particular mode. However, we do not know if this is helping to reduce the spread of *D. geminata*. The dispersal of *D. geminata* due to local vectors is continuing. New populations are being discovered regularly, within catchments and in new catchments. Regional groups are working well with central government to counteract this growing problem.

The regional approach to invasive species management needs to be more flexible, with increased funds available for quick responses to non-indigenous threats to the region. Central government has good structure to reduce invasions and response times for specific invasions. Nonetheless, it took one year to change the regulations regarding importation of equipment. Flexibility and quick response time is key when dealing with invasive species management.

The current punitive approach to *D. geminata* management, of issuing fines to individuals who spread *D. geminata*, does not seem to be working. This could be due to the difficulty in finding people to fine. Possibly the only way to get river and lake users to stop dispersing *D. geminata* and other aquatic species is to invoke a sense of responsibility, care and pride for the area that they use. River users would then endeavour not to spread the diatom. However, there will always be some percentage that will spread it without regard for the environment. This percentage of dispersers is the most destructive. Wild animals cannot be controlled so it is likely that there will always be the risk of some dispersal, but this is likely to be inconsequential compared to human vectors.

This study has helped to identify the problems and consequences of invasive species, but more research is needed. In particular, it should focus on a comparison of animal and human vectors, and the importance of continuing international dispersal of *D. geminata*.

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Appendix 1: Definitions

Appropriate Level of Protection: "the level of protection deemed appropriate by the member establishing a sanitary and phytosanitary measure to protect human, animal or plant life or health within its territory" (Wilson 2000).

Biosecurity: the protection of a country from biological impacts (Perrings *et al.* 2005).

Biotic invaders: species that establish a new range in which they proliferate, spread and persist to the detriment of the environment (Mack *et. al.* 2000).

Colonisation: described by Richardson *et al.* (2000) as plants in the founding population that reproduce and increase in number to form a colony which is self-perpetuating.

Dispersal: the process of movement of organisms between locations.

Introduction: the plant (or propagule) has been transported by humans across a major geographical barrier (Richardson *et al.* 2000).

Introduced or casual species: species found outside control or captivity as a potentially self-sustaining population (Williams and Fitter 1996). *Non-indigenous species* are those that are not originally from the area of interest, they could be from a different continent or the same continent (Rew *et al.* 2006).

I**nvasion process:** requires that introduced plants produce reproductive off-spring in areas distant from sites of introduction (Richardson 2000).

Minimum Viable Population: the relationship between a population's size and its chances of extinction (Shaffer 1981).

Mode: the manner or conveyance or both by which species are carried along a pathway (Mack 2003).

Naturalisation: species establishing new self-perpetuating populations. These colonies undergo widespread dispersal and become incorporated with the resident flora and fauna (Richardson *et. al.* 2000).

Pathway: the advance or progression in a particular direction regardless of the mode that disperses plants along that pathway (Mack 2003).

Propagule pressure: the composite measure of the number of individuals released into a region to which they are not native (Von Holle and Simberloff 2005).

Risk: the likelihood of an event occurring (Robertson 2000).

Risk assessment: the likelihood and severity of potential adverse effects of exposure to hazardous agents or activities (Andersen *et al.* 2004).

Risk management: the process of identifying, evaluating, selecting and implementing actions to reduce risk (Andersen *et al.* 2004).

Vector: how a species is transported, that is the physical means or agents (Ruiz and Carlton 2003).

Appendix 2: Statistical Analysis

> anova(pl.glm2, test = 'F')

Analysis of Deviance Table

Model: quasibinomial, link: logit

Response: cbind(live, dead)

Terms added sequentially (first to last)

Signif. codes: 0 â€˜***â€™ 0.001 â€˜**â€™ 0.01 â€˜*â€™ 0.05 â€˜.â€™ 0.1 â€˜ â€™ 1

Fitted model:
Percentage survival = mu + Time + Temp + Light + Wetness +
all two way interactions + all three way interactions +
four way interaction + error

Essentially, anything having stars (*) next to it will be statistically significant, at the 0.05 (*), 0.01 (**) and 0.001 (***) level.

+ Significant terms:
Time, temperature, light level and wetness.

+ Significant two-way interactions:
Time with temperature, time with light level, temperature with light level, time with wetness, temperature with wetness and light level with wetness.

	Df	Deviance	Resid. Df	Resid. Dev	F	Pr(>F)	
NULL			887	94090			
Time	1	14896	886	79194	207.7160	< 2.2e-16	***
Temp	3	25630	883	53564	119.1270	< 2.2e-16	***
Light.level	3	4229	880	49335	19.6578	2.556e-12	***
Wetness	1	2659	879	46676	37.0741	1.736e-09	***
Time:temp	3	12314	876	34362	57.2357	< 2.2e-16	***
Time:light.level	3	698	873	33664	3.2443	0.0214710	*
Temp:light.level	7	1321	866	32343	2.6314	0.0107941	*
Time:wetness	1	2025	865	30318	28.2427	1.375e-07	***
Temp:wetness	3	1311	862	29007	6.0915	0.0004219	***
Light.level:wetness	3	820	859	28187	3.8091	0.0099465	**
Time:temp:light.level	7	913	852	27274	1.8187	0.0805002	.
Time:temp:wetness	3	2116	849	25159	9.8338	2.231e-06	***
Time:light.level:wetness	3	274	846	24885	1.2713	0.2830233	
Temp:light.level:wetness	7	620	839	24265	1.2353	0.2804462	
Time:temp:light.level:wetness	7	78	832	24187	0.1553	0.9932459	

```
All these combinations are calculated across all times with assessments

Mean Survival by temperature (across light and wetness conditions)
  temp  percent   Stderr
4    5 72.64093 1.879033
1   12 65.19011 2.638818
2   20 51.28102 2.511393
3   28 24.51996 4.255230

Mean Survival by light (across temperature and wetness conditions)
  light.level  percent   Stderr
1   blueshade 72.86663 3.015851
2        dark 45.08180 2.689688
3       light 62.97938 2.498196
4      medium 62.57569 2.480610

Mean Survival by wetness (across temperature and light conditions)
  wetness  percent   Stderr
1    damp 53.18522 2.089195
2     wet 65.32579 1.730415

Mean Survival for combinations of conditions. Example: for 12 degrees, damp
conditions
and blueshade the average survival is 73%
   temp wetness light.level  percent    Stderr
21    5    damp   blueshade 70.67707  5.993133
22    5    damp        dark 56.93393  6.614096
23    5    damp       light 66.93718  6.693104
24    5    damp      medium 70.56956  6.007726
25    5     wet   blueshade 82.97053  3.139735
26    5     wet        dark 69.27398  5.365677
27    5     wet       light 81.72568  2.587931
28    5     wet      medium 82.03952  3.041792
1    12    damp   blueshade 63.22907  8.342052
2    12    damp        dark 52.55834  8.346005
3    12    damp       light 60.14914  8.537896
4    12    damp      medium 66.31380  8.098374
5    12     wet   blueshade 73.00697  6.104617
6    12     wet        dark 49.32764  8.197202
7    12     wet       light 85.81091  3.007635
8    12     wet      medium 71.12497  5.886853
9    20    damp        dark 39.11581  6.434477
10   20    damp       light 41.84208  6.546671
11   20    damp      medium 44.92467  6.521290
12   20     wet        dark 30.08174  5.689369
13   20     wet       light 76.81833  3.629162
14   20     wet      medium 74.90347  3.873369
15   28    damp        dark 22.54058 10.227361
16   28    damp       light 27.46493 10.951874
17   28    damp      medium 25.84587 10.808400
18   28     wet        dark 20.57003 10.327457
19   28     wet       light 25.89636 10.930719
20   28     wet      medium 24.80196 10.994116
```